STORED-GRAIN INSECT MANAGEMENT WITH INSECTICIDES: EVALUATION OF EMPTY- BIN AND GRAIN TREATMENTS AGAINST INSECTS COLLECTED FROM KANSAS FARMS

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Abstract

The insecticides, β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin, are approved in the United States for treating empty bin surfaces. Chlorpyrifos-methyl plus deltamethrin and spinosad insecticides are approved for direct treatment of wheat. The efficacy of commercial formulations of β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin at labeled rates was evaluated against adults of 16 field strains of the red flour beetle, *Tribolium castaneum* (Herbst); seven strains of sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and two strains of the lesser grain borer, *Rhyzopertha dominica* (F.). Concrete arenas in plastic Petri dishes (9 cm diameter) were used to simulate the concrete floor of empty bins. The time for ~100% knockdown and mortality of adults of laboratory strains of the three species was first established by exposing them to insecticide-treated concrete surfaces for 1 to 24 h. Adults of field strains of each species were exposed to specific established insecticide-time combinations. Mortality of all species was lower than knockdown, suggesting recovery after seven days when placed on food. Chlorpyrifos-methyl plus deltamethrin did not control all *R. dominica* and most *O. surinamensis* field strains. β-cyfluthrin was extremely effective against *R. dominica* but ineffective against *T. castaneum* and *O. surinamensis* field strains, even at four times the high labeled rate.

Field strains of *R. dominica* were highly susceptible to spinosad and chlorpyrifos-methyl plus deltamethrin at labeled rates on hard red winter wheat. Strains of *T. castaneum* and *O. surinamensis* were susceptible only to the latter insecticide. Dose-response tests with spinosad on the two least susceptible field strains of each species showed the lethal dose for 99% mortality (LD_{99}) for *T. castaneum* and *R. dominica* field strains were similar to that of the corresponding laboratory strains. Corresponding values for the two *O. surinamensis* field strains were

significantly greater (~6 times) than the laboratory strain. The effective dose for progeny reduction (ED_{99}) of only one *R. dominica* field strain was significantly greater (~2 times) than that of the laboratory strain. The baseline susceptibility data of field strains of three insect species to spinosad will be useful for monitoring resistance development once this product is commercially released as a grain protectant.

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Chapter 1 - Variation in susceptibility of field strains of three stored-grain insect species to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces

Abstract

The efficacy of commercial formulations of β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin as surface treatment was evaluated against adults of 16 field strains of the red flour beetle, *Tribolium castaneum* (Herbst); seven to eight strains of sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and two strains of the lesser grain borer, *Rhyzopertha dominica* (F.) by studying the time-dependent knockdown, 7-d mortality, and progeny production. Time-mortality responses of field-collected strains were compared with adults of corresponding laboratory strains that had been reared on standard diets since 1999. The time for ~100% knockdown and mortality of laboratory strains of the three species was first established by exposing them to insecticide-treated concrete surfaces for 1 to 24 h. Linear or nonlinear models fitted to knockdown and mortality data over time showed significant differences among insecticides and species. Mortality of all species was less than that of knockdown, suggesting recovery when placed on food. Chlorpyrifos-methyl plus deltamethrin did not control all *R. dominica* and most *O. surinamensis* field strains. β-cyfluthrin was extremely effective against *R. dominica* but ineffective against *T. castaneum* and *O. surinamensis* field strains as evidenced by low mortality and high progeny production. Exposing the two least susceptible field strains of *O. surinamensis* and three of *T. castaneum* to one to four times the high labeled rate of β-cyfluthrin resulted in only 36 to 90% mortality Reduced susceptibility in field strains may be due to inherent formulation deficiency or low levels of tolerance or resistance to β-cyfluthrin.

KEY WORDS stored-grain insects, β-cyfluthrin, chlorpyrifos-methyl plus deltamethrin, field strains, efficacy assessment

Introduction

Stored grain insect management, prior to storing newly harvested grain, begins with cleaning empty bins by removing residual grain debris and application of an approved insecticide to the concrete floor and interior bin surfaces to kill any live insects present. This practice is followed by 78.8% of the 318 Kansas producers storing wheat who were surveyed (Reed and Pedersen 1987). Insecticides approved by the United States Environmental Protection Agency (US-EPA) for treating empty bins and grains are shown in Table 1.1. Among the insecticides registered for empty bin treatments, β-cyfluthrin or Tempo® SC Ultra (Bayer CropScience, Research Triangle Park, NC, USA) is new, and is an alternative to traditionally used cyfluthrin wettable powder and emulsifiable concentrate formulations. β-cyfluthrin can be applied to surfaces at low and high application rates of 0.01and 0.02 g (AI)/m^2, respectively. These rates are 50% less than that of the wettable powder or emulsifiable concentrate formulations. Chlorpyrifos-methyl plus deltamethrin was registered by the US-EPA on 27 October 2004 for use on barley, oats, rice, sorghum, and wheat as well as empty bins receiving these grains. This combination product replaced chlorpyrifos-methyl (Reldan 4E) used at 6 mg(AI)/kg after the product registrants (Dow AgroSciences, Indianapolis, IN and Bayer CropScience, Research Traingle Park, NC) petitioned US-EPA to revoke existing tolerances and cancel product registrations, which became effective 5 December 2007 (Anonymous 2007).

The efficacy of wettable powder and emulsifiable concentrate formulations of cyfluthrin at low (0.02 g [AI]/m^2) and high (0.04 g [AI]/m^2) label rates was evaluated on concrete surfaces against laboratory populations of the red flour beetle, *Tribolium castaneum* (Herbst); confused flour beetle, *Tribolium confusum* (Jacquelin du Val); and Indianmeal moth, *Plodia interpunctella* (Hübner), (Arthur 1994, 1998a,b; 1999a,b). The wettable powder formulation was found to be more effective than the emulsifiable concentrate formulation, probably due to greater availability of residues on treated surfaces (Parkin 1966). Additionally, the wettable powder formulation was more persistent than the emulsifiable concentrate formulation on both steel (Arthur 1992) and concrete (Arthur 1994). Exposure of *T. castaneum* adults to low rate of cyfluthrin wettable powder on concrete for 0.5 to 4 h in the absence of flour resulted in 100% mortality; similar mortality at the high rate occurred only after 2 h of exposure (Arthur 1998a). If *T. castaneum* adults were provided 1 g of flour for 1 wk after a 2 h of exposure to low rate of cyfluthrin

wettable powder, 40% of the adults recovered (Arthur 1998b). Arthur (2000) reported that *T. castaneum* adults exposed for 2 h on concrete treated with low rate of cyfluthrin wettable powder and then transferred to concrete dishes for 1 wk showed 60% mortality in the absence of food, 49% in presence of 1 g of wheat kernels, 15% in 1 g of sawdust, and 5% in 1 g of flour. Differences in insect responses in the presence and absence of food between the Arthur (1998b) and Arthur (2000) could be attributed to temperatures used. Arthur (1998b) conducted tests at 22°C and Arthur (2000) at 28°C. Pyrethroids such as cyfluthrin are known to have negative temperature coefficient, and the toxicity of cyfluthrin to *T. castaneum* adults was found to decrease markedly at 25, 30 and 35°C when compared with 20°C (Arthur 1999c). β-cyfluthrin was found to be effective against the stored-product psocid species on concrete at the rate of 2.4 mg (AI)/m^2 at 30°C and 70% RH (Guedes et al. 2008). The efficacy of β-cyfluthrin against stored-grain insects other than psocids needs to be confirmed.

Chlorpyrifos-methyl plus deltamethrin was effective against several stored-grain psocids on stored wheat (Athanassiou et al. 2009). It was also effective against the lesser grain borer, *Rhyzopertha dominica* (F.); rice weevil, *Sitophilus oryzae* (L.); *T. castaneum*, and *P. interpunctella* on stored wheat and against *R. dominica* and *S. oryzae* on short-grain and long-grain rices (Subramanyam et al. 2012), but data on its efficacy as surface treatment are lacking.

All of the studies mentioned above were conducted using laboratory reared stored-grain insects. Field collected insects may differ widely in their susceptibility to insecticide applied to empty bins or to grains due to natural tolerance or resistance (Subramanyam et al. 1989, Subramanyam and Hagstrum 1996, Huang et al. 2004, Kljajic and Peric 2006, Athanassiou et al. 2008). To date, there are no published studies documenting effectiveness of β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin on empty bin surfaces against field strains of stored-grain insect populations. Such an evaluation is necessary to confirm whether or not an approved insecticide will work in practical field situations at the labeled rates. Most grain bins have concrete floors. Therefore, in the present investigation, we determined susceptibility of adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains collected mostly from Kansas farm-stored grain and mills and strains from other locations of USA to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin applied to concrete surfaces in the laboratory.

Materials and Methods

Collection of Field Strains. Cooperating farm sites (Table 1.2) were visited one to three times between July and November 2011 to collect adults of *T. castaneum, R. dominica* and *O. surinamensis* from farm bins by inserting five perforated probe traps (Subramanyam et al.1993) just below the grain surface to capture live adults of insect species. These traps were removed after 1 to 2 wk. Additionally, 1 to 2 kg sample of mostly wheat, and some corn and sorghum, were collected in 30.5 cm wide and 37.5 cm long plastic Ziploc bags (Assorted Bag Company, Dallas, TX). In the laboratory, 2.38-mm diameter aluminum sieves and pans (Seedburo Equipment Company, Des Plaines, IL, USA) were used to separate live adults of insects from grains. In addition, five strains of *T. castaneum* and one strain of *R. dominica* collected from flour mills in the USA prior to 2011 were also included in this study, along with the laboratory strains of each species, that have been in rearing, without insecticide exposure, since 1999 in the Department of Grain Science and Industry, Kansas State University. These laboratory strains served as the standard reference strains and assumed to be insecticide-susceptible.

Insect Rearing. Laboratory and field strains were reared on standard diets in a growth chamber at 28°C and 65% RH. Organic white wheat flour (Heartland Mills, Marienthal, KS, USA) plus 5% (by wt) brewer's yeast diet was used for rearing *T. castaneum*, while clean organic hard red winter wheat (Heartland Mills) and rolled oats plus 5% brewer's yeast diet were used for rearing *R. dominica* and *O. surinamensis*, respectively.

Concrete-Poured Petri Dishes. Ready-mix concrete (Rockite, Hartline Products Co., Inc., Cleveland, OH, USA) was mixed with tap water to make a slurry. This slurry was poured into 9-cm diameter, 1.5 cm high and 62 cm^2 area plastic petri dishes (Fisher Scientific, Denver, CO, USA). 3,810 g of concrete was mixed with 1,905 ml of tap water to pour 100 dishes. The slurry was allowed to dry and the inside walls of the petri dishes were coated with polytetrafluoroethylene (Insecta-a-Slip, Bio Quip Products, Inc., Rancho Dominguez, CA, USA) to prevent insects from crawling on the sides of dishes.

Treatment of Concrete Dishes with Insecticides. β-cyfluthrin (11.8% purity) and chlorpyrifos-methyl plus deltamethrin (21.6 and 3.7% purity), were supplied by Bayer CropScience (Research Traingle Park, Raleigh, NC)and were diluted in water. Concrete surfaces of dishes were treated with β-cyfluthrin at the low labeled rate of 0.01 and the high labeled rate

of 0.02 g (AI)/m^2, and with chlorpyrifos-methyl plus deltamethrin at the labeled rate of 0.12 plus 0.02 g (AI)/m^2 by applying 255 µl spray solution per dish using a Badger 100 artist's airbrush (Model 100, Franklin Park, IL, USA). Dishes sprayed with 255 µl of distilled water served as the control treatment. Treated dishes were allowed to dry under room conditions (25°C and 25% RH) for 24 h before exposing insects.

Time-response tests with laboratory strains. The laboratory strains of *T. castaneum*, *O. surinamensis*, and *R. dominica* were used to establish a time at which 100% or close to 100% knockdown and mortality of adults occurred when exposed to labeled rates of β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin. This time was used to expose field strains of each species to concrete treated with the two insecticides. Ten unsexed (1- to 2-wk-old) adults each of *T. castaneum*, *O. surinamensis*, or *R. dominica* from laboratory cultures were introduced into each dish and the dishes were covered with Petri dish lids. Adults were exposed to treated dishes for 1, 2, 4, 8, 12, 16, 20 and 24 h. Separate dishes were used for each time period. In addition, each exposure time and species included an untreated control dish which was treated with aliquots of distilled water. Each species, insecticide, rate, and time combination, including the control treatment, was replicated three times. Dishes were arranged on a laboratory table and HOBO® data loggers (Onset Computer Corp., Bourne, MA, USA) indicated the mean ± SE temperature and relative humidity during insect exposure to be 24.3 ± 0.04°C and 23.4 ± 0.06% RH.

At each exposure time, adults that were knocked down and active were counted. After counting, all adults were transferred to 150-ml round plastic containers with 30 g of the respective insect diet. The plastic containers had perforated lids with wire-mesh screens to facilitate air diffusion. Containers were incubated at 28°C and 65% RH for 1 wk to determine end-point mortality following insect recovery on diets. After assessing mortality, the adults were discarded and diets were placed back in the 150 ml containers and held at 28°C and 65% RH for an additional 35 d to determine adult progeny production from eggs laid by parental adults during the 1 wk recovery period.

Exposure of field strains at labeled rates for a fixed time. The time at which the knockdown and mortality of adults was 100% or near 100% for a given species and insecticide combination was used to expose adults of field strains of stored grain insect species collected from Kansas farms. Adults (10) of field strains of each insect species, along with the corresponding laboratory strain, were tested following protocols mentioned above. The low

labeled rate of β-cyfluthrin gave poor control of *T. castaneum* and *O. surinamensis* laboratory strains with mortalities of 46.7 ± 6.7 and 83.3 ± 8.8% at the maximum exposure time of 24 h, respectively. Therefore, adults of these two species were exposed for 24 h to concrete treated only with the high labeled rate of β-cyfluthrin, while *R. dominica* was exposed to this insecticide for 2 h because of its susceptibility. All three species were exposed for 8 h to concrete treated with chlorpyrifos-methyl plus deltamethrin. Each insecticide, species, and strain combination was replicated five times. Knockdown, mortality, and adult progeny production of field strains were determined as explained above. Tests with field strains of the three species and the laboratory strains were performed at room conditions where mean ± SE temperature and relative humidity were 25.4 ± 0.02°C and 17.2 ± 0.1%, respectively, and also at constant conditions in a growth chamber at 28°C and 65% RH. At room conditions, low mortality was observed, so the tests were also done at constant conditions to see if there was any temperature effect as these insecticides are applied to empty bins 3 to 4 wk prior to storing new grain after harvest during the summer months of June and July. At room conditions, each strain had its respective control treatment. At constant conditions only the laboratory strain served as the control for all strains because knockdown and mortality of field strains in the control treatments in tests at room conditions were less than 10%.

Dose-response tests with β-cyfluthrin on laboratory and least susceptible field strains. Based on field strain responses to insecticides, three least susceptible strains of *T. castaneum* and two of *O. surinamensis*, along with corresponding laboratory strains, were exposed to β-cyfluthrin-treated concrete dishes at one to four times the high labeled rate (0.02 to 0.08 g [AI]/m^2) to assess knockdown, mortality, and progeny production. Dishes sprayed with distilled water served as the control treatment for all strains. All dose-response tests were performed at 28°C and 65% RH. There were five replications for each species, strain, and β-cyfluthrin rate, and 10 adults were exposed in each replication.

Data Analysis. Adults of all species that were knocked down and those that died after 1 wk recovery on diets out of the total exposed in an individual Petri dish were calculated as a percentage. In the time-response tests with the laboratory strains, there was no knockdown and mortality in the control treatment for *T. castaneum* and *R. dominica* during 1 to 24 h exposures. The maximum mean ± SE knockdown of *O. surinamensis* in the control treatment was 3.3 ± 3.3% and mortality was 10.6 ± 0.6%. Therefore, knockdown and mortality data were not

corrected for these responses in the control treatment. Linear ($y = a + bx$) or nonlinear models ($y = a + b/x^2$) were fit to knockdown and mortality responses over time for each species of laboratory strains by insecticide and rate using Table curve 2D software (Jandel Scientific, San Rafael, CA, USA). No model could be fit when knockdown or mortality was 100% at all exposure times; for example this occurred with *R. dominica* mortality and with *O. surinamensis* knockdown at both rates of β-cyfluthrin. Linear or nonlinear models fit to data allowed for statistical comparison of knockdown and mortality responses for each insect species and insecticide, and for comparison of knockdown or mortality responses between insecticides, between the two β-cyfluthrin rates, and between species by insecticide and rate. These pair-wise comparisons involved comparing individual models fit to data (SAS Institute 2008) with a pooled model fit to pooled data of the pairs being compared (Draper and Smith 1981). Individual models were considered significantly different ($P \leq 0.05$) from one another if the *F*-test showed the individual models deviated significantly from the pooled model.

The mean ± SE knockdown and mortality of all *T. castaneum, O. surinamensis* and *R. dominica* field strains in the control treatment at room conditions ranged from 0 to 2.0 ± 2.0%, 0 to 6.7 ± 6.7%, and 0 to 4.5 ± 2.8%, respectively. So the knockdown and mortality data of field strains at room conditions was not corrected for these responses in the control treatment. At constant conditions, the mean ± SE mortality of *T. castaneum* in the control treatment was 0%, but that of *O. surinamensis* and *R. dominica* was 14.2 ± 4.0% and 20.9 ± 8.2%, respectively. Therefore, the mortality data of *R. dominica* and *O. surinamensis* at constant conditions were corrected for control mortality (Abbott 1925). The knockdown and mortality data of field strains at room and constant environmental conditions at established fixed times were analyzed by species after transformation to angular values (Zar 1984) for normalizing heteroscedastic treatment variances. Knockdown or mortality data were subjected to two-way analysis of variance (ANOVA) to determine differences ($P \leq 0.05$) between insecticides and among strains. For each insecticide and species, knockdown and mortality data were analyzed by one-way ANOVA and Dunnett's procedure was used to determine if responses of each field strain differed from that of the corresponding laboratory strain (SAS Institute 2008).

In dose-response tests with β-cyfluthrin at constant conditions, knockdown and mortality responses of *T. castaneum* and *O. surinamensis* strains were not corrected for the corresponding control responses as the mean ± SE knockdown was 0% and mortality ranged from 0 to 8.1 ±

2.7% for strains of both species. Knockdown or mortality data for each species by strain were subjected to one-way ANOVA, and the least squares means test was used to determine differences ($P \leq 0.05$) among the four β-cyfluthrin rates.

Progeny production data in the control and insecticide treatments were not analyzed statistically, because progeny data did not follow any trends as unsexed adults were used in tests. Additionally, there were variations in progeny production within and among strains in different tests. Therefore, only means and associated standard errors were calculated for progeny production data.

Results

Time-Mortality Responses of Laboratory Strains. The mean ± SE knockdown of *T. castaneum* adults during 1 to 24 h exposures ranged from 20.0 ± 11.6 to 100.0% at the low β-cyfluthrin rate and 43.3 ± 18.6 to 100.0% at the high labeled rate (Fig. 1.1A). Mortality of *T. castaneum* adults at the low and high β-cyfluthrin rate increased linearly with time but never reached 100% (Fig. 1.1B). The increase in knockdown and mortality of *T. castaneum* adults with time when exposed to chlorpyrifos-methyl plus deltamethrin treated concrete surfaces was non-linear and 100% knockdown and mortality were achieved at 4 h and 8 h, respectively. Knockdown of *O. surinamensis* exposed to β-cyfluthrin was 100% at all times when exposed to both the low and high labeled rates (Fig. 1.1C). Adult mortality increased in a non-linear fashion and failed to reach 100% at the low rate but reached 100% only with the high rate at 24 h (Fig. 1.1D). Complete knockdown of *O. surinamensis* adults occurred at 8 h (Fig. 1.1C) and complete mortality at 4 h (Fig. 1.1D) on chlorpyrifos-methyl plus deltamethrin-treated concrete. Unlike *T. castaneum* and *O. surinamensis*, *R. dominica* was extremely susceptible to β-cyfluthrin and all adults died at low and high β-cyfluthrin rates, irrespective of the exposure time (Fig. 1.1F). However, with chlorpyrifos-methyl plus deltamethrin, mean ± SE knockdown and mortality ranged from 16.7 ± 3.3 to 100.0 ± 0.0% (Fig. 1.1E) and 7.4 ± 7.4 to 100.0 ± 0.0% (Fig. 1.1F), respectively, at all exposure times. Except for *R. dominica* adults exposed to β-cyfluthrin, adults of *T. castaneum* and *O. surinamensis* exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin recovered when placed on diets. The degree of recovery varied with the exposure time and the insecticide used.

Knockdown and mortality responses for each insect species-insecticide combination were described by linear or nonlinear models (r^2 = 0.80 – 0.99) (Fig. 1.1A-F), and the model parameters are given in Table 1.3. Knockdown responses over time of *T. castaneum* exposed to β-cyfluthrin low and high rate were nonlinear, whereas mortality responses at each of the rates were linear. Therefore, for β-cyfluthrin, at each rate, statistical comparison between knockdown and mortality was not possible. Comparisons between knockdown and mortality at each β-cyfluthrin rate could not be made because of 100% mortality of *R. dominica* or 100% knockdown of *O. surinamensis* at all exposure times. Knockdown and mortality responses of *T. castaneum*, *R. dominica*, or *O. surinamensis* adults exposed to chlorpyrifos-methyl plus deltamethrin were not significantly different from one another (F, range among species = 0.94 - 3.30; df = 2, 12; P = 0.072 - 0.417).

β-cyfluthrin at the high rate caused significantly greater knockdown of *T. castaneum* adults than β-cyfluthrin low rate and chlorpyrifos-methyl plus deltamethrin (Table 1.4). Knockdown responses of *R. dominica* adults at β-cyfluthrin low and high rate were essentially similar, but at each rate the knockdown responses were significantly greater than that of chlorpyrifos-methyl plus deltamethrin. The high rate of β-cyfluthrin caused significantly greater mortality of *O. surinamensis* adults when compared with the low rate.

Knockdown responses of *R. dominica* exposed to β-cyfluthrin at the low or high rate were greater than that of *T. castaneum* (Table 1.5). Knockdown responses of *O. surinamensis* exposed to chlorpyrifos-methyl plus deltamethrin were greater when compared with that of *T. castaneum* or *R. dominica*. Similarly, mortality responses of *O. surinamensis* were significantly greater than that of *R. dominica* but not *T. castaneum*.

Adult Progeny Production of Laboratory Strains. Mean ± SE progeny production of *T. castaneum* at 1 to 24 h exposures in the control treatment ranged from 218.3 ± 10.8 to 571.3 ± 48.8 adults (Table 1.6). The mean reduction in *T. castaneum* adult progeny production at the low and high β-cyfluthrin rate relative to the control treatment was 62 to 96%. Adults of *T. castaneum* exposed to chlorpyrifos-methyl plus deltamethrin for 8 to 24 h failed to produce progeny, because of 100% mortality of exposed adults. However, at exposure times of 1 to 4 h, reduction in progeny production relative to the control treatment was 23 to 99%.

In the control treatment, at all exposure times, *O. surinamensis* progeny production ranged from 137.3 ± 29.7 to 233.0 ± 46.1 adults (Table 1.7). The reduction in progeny

production at both β-cyfluthrin rates was 83 to 100%. The reduction in progeny production of *O. surinamensis* exposed for 1 to 2 h to chlorpyrifos-methyl plus deltamethrin was 36 to 77%, whereas those exposed for 4 to 24 h was 99 to 100%.

Adult progeny production of *R. dominica* at 1 to 24 h exposures in the control treatment ranged from 43.7 ± 23.0 to 174.7 ± 19.2 adults (Table 1.8). No progeny were produced by *R. dominica* adults exposed to β-cyfluthrin, because of 100% mortality. More adult progeny were produced by *R. dominica* exposed for 1 to 4 h to chlorpyrifos-methyl plus deltamethrin, but the reduction in progeny production ranged from 85 to 100% for those exposed for 8 h or greater.

Responses of Field Strains at Room Conditions. The mean ± SE knockdown of all *T. castaneum* field strains exposed to the high rate of β-cyfluthrin-treated concrete ranged from 89.8 ± 3.2 to 98.0 ± 2.0%, and mean ± SE mortality ranged 16.0 ± 6.8 to 67.1 ± 15.6% (Fig. 1.2A) with recovery on diet ranging from 31.5 to 83%. One-way ANOVA by insecticide used showed that knockdown among *T. castaneum* strains exposed to the high rate of β-cyfluthrin was not significant (F= 1.56; df = 16, 73; P = 0.101), but mortality differences among strains were significant (F= 2.14; df = 16, 73; P = 0.015). Although ANOVA showed mortality differences among strains, Dunnett's test (SAS Institute 2008) showed that the mortality of each strain did not differ significantly (P > 0.05) from that of the laboratory strain. The mean ± SE progeny production of the 16 field strains and the laboratory strain in the control treatment ranged from 306.8 ± 35.0 to 541.0 ± 14.5 adults, whereas on β-cyfluthrin-treated concrete it ranged from 77.4 ± 36.9 to 276.0 ± 20.7 adults (Table 1.9).

The mean ± SE knockdown of five of the seven *O. surinamensis* field strains exposed to β-cyfluthrin was 100%, whereas it ranged from 71.3 ± 5.0 to 75.6 ± 10.1% for the other two field strains (Fig. 1.2B). The mean ± SE mortality for the five strains that showed 100% knockdown was 86.0 ± 4.0 to 100%, and it ranged from 35.9 ± 3.7 to 48.9 ± 13.5% in the other two strains, indicating recovery when placed on diet. Strains of *O. surinamensis* exposed to β-cyfluthrin differed from each other significantly in knockdown (F= 17.71; df = 7, 32; P < 0.0001) and mortality (F= 13.98; df = 7, 31; P < 0.0001). Knockdown and mortality responses of two strains differed significantly from that of the laboratory strain (P < 0.05; Dunnett's test). The mean ± SE progeny production of all strains in the control treatment ranged from 7.0 ± 3.4 to 168.7 ± 21.6 adults, whereas those exposed to β-cyfluthrin ranged from 0 to 33.8 ± 14.6 adults (Table 1.9).

β-cyfluthrin was extremely effective against *R. dominica* field strains with more than 98% knockdown and 100% mortality. Knockdown responses among strains exposed to the high rate of β-cyfluthrin were not significantly different from one another (F= 1.0; df = 2, 14; P = 0.397) (Fig. 1.2C). The progeny production of all strains in the control treatment ranged from 68.6 ± 15.1 to 115.4 ± 21.3 adults, and there was complete progeny suppression of both field strains exposed to β-cyfluthrin.

The knockdown of 11 out of 16 *T. castaneum* field strains exposed to chlorpyrifos-methyl plus deltamethrin was greater than 90%, and only eight strains had mortality greater than 90% (Fig. 1.2D). Mortality was less than 50% in two field strains and the overall recovery on diet ranged from 0 to 50%. One-way ANOVA showed that the field strains differed significantly among each other in knockdown (F= 4.60; df =16, 73; P < 0.0001) and mortality (F= 4.36; df = 16, 73; P < 0.0001). Knockdown response of one strain and mortality of two strains differed from that of the laboratory strain (P < 0.05; Dunnett's test). Progeny production among strains exposed to the chlorpyrifos-methyl plus deltamethrin -treated surfaces ranged from 0 to 146.8 ± 56.2 adults (Table 1.9).

The mean ± SE knockdown of seven *O. surinamensis* field strains exposed to chlorpyrifos-methyl plus deltamethrin ranged from 68.0 ± 8.6 to 96.0 ± 4.0%, and the mortality ranged from 38.2 ± 10.5 to 98.0 ± 2.0% with a recovery of 0 to 44% on diet (Fig. 1.2E). Field strains of *O. surinamensis* exposed to chlorpyrifos-methyl plus deltamethrin differed from each other in knockdown (F= 2.67; df = 7, 32; P = 0.027) and mortality (F= 5.33; df = 7, 31; P = 0.0004). Knockdown of one strain and mortality of two strains differed from that of the laboratory strain (P < 0.05; Dunnett's test). Progeny production among strains exposed to chlorpyrifos-methyl plus deltamethrin treated surfaces ranged from 0 to 19.8 ± 8.1 adults (Table 1.9).

The two field strains of *R. dominica* showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin (Fig. 1.2F), because knockdown ranged from 84.3 ± 2.7 to 89.8 ± 3.2% and mortality from 7.3 ± 3.0 to 21.9 ± 8.3%. The recovery of the two field strains on diet ranged from 74 to 92%. One-way ANOVA showed that the knockdown (F= 17.31; df = 2, 12; P = 0.0003) and mortality (F= 21.31; df = 2, 12; P = 0.0001) responses of laboratory and two field strains differed significantly from each other. Mortality responses of both the field strains

differed significantly from that of the laboratory strain ($P < 0.05$; Dunnett's test). Field strains exposed to chlorpyrifos-methyl plus deltamethrin produced 73.6 ± 20.4 to 83.0 ± 25.5 adults.

Responses of Field Strains at Constant Conditions. β-cyfluthrin was less effective against *T. castaneum* field strains at constant conditions when compared with room conditions since knockdown was less than 90% in five of the 16 field strains and mortality was less than 51% in all strains including the laboratory strain (Fig.1.3A). In contrast, at room conditions knockdown of all strains was more than 90% and mortality of only 11 strains was less than 50%. There were significant differences among *T. castaneum* strains exposed to β-cyfluthrin in knockdown ($F = 2.14$; df = 16, 68; $P = 0.016$) and mortality ($F = 2.26$; df = 16, 68; $P = 0.011$). Progeny production of the laboratory strain in the control treatment was 152.2 ± 27.2 adults, and the 16 field strains exposed to β-cyfluthrin produced 19.2 ± 7.8 to 216.2 ± 50.4 adults (Table 1.10).

The knockdown of six of eight *O. surinamensis* field strains (one extra strain than those tested at room conditions) exposed to β-cyfluthrin was greater than 94% and for the other two it was 52.7 ± 6.9 to 58.3 ± 7.5% (Fig. 1.3B). Mortality among the eight field strains ranged from 5.0 ± 3.6 to 81.7 ± 6.6% and the recovery on diet ranged from 18 to 90%. Knockdown ($F = 27.38$; df = 8, 36; $P < 0.0001$) and mortality ($F = 15.80$; df = 8, 35; $P < 0.0001$) responses among *O. surinamensis* strains exposed to β-cyfluthrin were highly significant. Knockdown of two strains and mortality of three strains were significantly different from that of the laboratory strain ($P < 0.05$; Dunnett's test). The progeny production of the laboratory strain in the control treatment was 142.6 ± 35.1 adults, and strains exposed to β-cyfluthrin produced 0.2 ± 0.2 to 47.2 ± 7.2 adults (Table 1.10).

β-cyfluthrin was extremely effective against the two *R. dominica* field strains and the laboratory strain with 98 to 100% knockdown and 100% mortality (Fig. 1.3C); similar responses were observed under room conditions. No progeny were produced when all three strains were exposed to β-cyfluthrin, and the laboratory strain in the control treatment produced 64.2 ± 17.1 adults (Table 1.10).

Chlorpyrifos-methyl plus deltamethrin was more effective against *T. castaneum* field strains at constant conditions than at room conditions, with 94.0 ± 2.4 to 100% knockdown. The mortality ranged from 90.0 ± 3.2 to 100% among the strains (Fig.1.3D). Field strains of *T. castaneum* exposed to chlorpyrifos-methyl plus deltamethrin differed from each other

significantly in knockdown (F = 1.82; df = 16, 68; P = 0.047) and mortality (F = 3.93; df = 16, 68; P < 0.0001). The knockdown and mortality of one strain differed significantly from that of laboratory strain (P < 0.05; Dunnett's test). There was no progeny production in 15 field strains and one strain produced just 3.0 ± 3.0 adults (Table 1.10).

The knockdown and mortality of the eight *O. surinamensis* field strains exposed to chlorpyrifos-methyl plus deltamethrin ranged from 77.1 ± 8.4 to 91.6 ± 4.1% and 66.6 ± 5.8 to 97.7 ± 2.3%, respectively (Fig. 1.3E). Knockdown (F = 2.41; df = 8, 36; P = 0.034) and mortality (F = 3.29; df = 8, 36; P = 0.007) responses were highly significant among the strains. Dunnett's test showed that none of the strains is different in both knockdown and mortality from the laboratory strain. The progeny produced among the strains exposed to chlorpyrifos-methyl plus deltamethrin was negligible, ranging from 0 to 4.0 ± 2.3 adults (Table 1.10).

Chlorpyrifos-methyl plus deltamethrin produced higher knockdown (98.0 ± 2.0 to 98.6 ± 1.4%) and mortality (38.2 ± 14.5 to 40.2 ± 3.9%)in the two *R. dominica* field strains at constant conditions than at room conditions (Fig. 1.3F). The three strains of *R. dominica* exposed to chlorpyrifos-methyl plus deltamethrin differed significantly in mortality (F= 17.10; df = 2, 12; P = 0.0003) but not in knockdown (F= 0.51; df = 2, 12; P = 0.614). Only mortality responses of the two field strains were significantly different from that of the laboratory strain (P < 0.05; Dunnett's test). No progeny was produced by the laboratory strain exposed to chlorpyrifos-methyl plus deltamethrin and approximately 17 adult progeny were produced by each field strain (Table 1.10).

Dose-Response Tests with β-cyfluthrin. Exposing the three least susceptible strains of *T. castaneum* (CF, PD, and TP) to up to 4 times the high rate of β-cyfluthrin resulted in 96.0 ± 2.4 to 100% knockdown and 54.0 ± 9.3 to 90.0 ± 7.7% mortality (Table 1.11). The knockdown and mortality of the corresponding laboratory strain at all rates of β-cyfluthrin ranged from 96.0 ± 2.4 to 100% and 72.0 ± 10.7 to 90.0 ± 7.7%, respectively. Except for the mortality of TP strain which was different among the four β-cyfluthrin rates (F= 5.55; df = 3, 16; P = 0.008), the knockdown (F, range among strains = 0.76 - 2.67; df = 3, 16; P = 0.083 - 0.532) and mortality (F, range among strains = 0.91 - 2.11; df = 3, 16; P = 0.139 - 0.459) responses of all strains were similar among β-cyfluthrin rates. In the TP strain, mortality at rates 0.04 to 0.08 g (AI)/m^2 was similar (P > 0.05), and mortality at rates of 0.04 and 0.08 g (AI)/m^2 was significantly greater (P < 0.05) than at 0.02 g (AI)/m^2.

There was complete knockdown and mortality of the laboratory strain of *O. surinamensis* at all β-cyfluthrin rates. The knockdown of field strains AB1 and AB2 among β-cyfluthrin rates was 71.3 ± 3.9 to 100% while the mortality was 36.0 ± 10.3 to 76.9 ± 9.3% (Table 1.11). Knockdown responses of the strains differed significantly among the rates of β-cyfluthrin (*F*, range among strains = 3.87 - 4.63; df = 3, 16; *P* = 0.016 - 0.03), but mortality responses were not different among rates (*F*, range among strains = 1.22 - 2.62; df = 3, 16; *P* = 0.087 - 0.336). In strain AB1, knockdown at rates of 0.04 to 0.08 g (AI)/m^2 was similar ($P > 0.05$) and knockdown at rates of 0.04 and 0.08 g (AI)/m^2 was significantly greater ($P < 0.05$) than at 0.02 g (AI)/m^2. In strain AB2 knockdown at rates of 0.04, 0.06 and 0.08 g (AI)/m^2 was similar and knockdown at these rates were all significantly greater ($P < 0.05$) than at 0.02 g (AI)/m^2.

Progeny production of the three field strains and the laboratory strain of *T. castaneum* that were not exposed to β-cyfluthrin (control) ranged from 287.0 ± 8.1 to 357.4 ± 42.4 adults, while those exposed to β-cyfluthrin ranged from 3.6 ± 2.4 to 89.6 ± 35.2 adults (Table 1.12). The progeny produced by the laboratory and field strains of *O. surinamensis* in the control treatment was 71.8 ± 24.4 to 100.4 ± 26.9 adults. Complete or near-complete suppression of progeny production was observed only with the laboratory strain because of 100% mortality of adults. Progeny production was observed in the field strains and the numbers produced decreased with an increase in β-cyfluthrin rate (Table 1.12).

Discussion

The field strains of *T. castaneum* and *O. surinamensis* were generally less susceptible to both chlorpyrifos-methyl plus deltamethrin and β-cyfluthrin residues on concrete surfaces. However, the field strains of *R. dominica* showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin but not to β-cyfluthrin. Variation in susceptibility of different insect species and strains to insecticides could be due to the bioassay technique used, natural tolerance, and/or resistance (Subramanyam and Hagstrum 1996). Collins and Wilson (1987) compared two different bioassay methods, filter paper and treated grain assays, against same strains of *O. surinamensis* and found that the resistance ratios for pyrethroids from both assays were not correlated. The probit regression slopes were steeper for grain assays than filter paper assays. Field strains of the granary weevil, *Sitophilus granarius* (L.), from different locations in the

former Yugoslavia were 0.5 to 30 times less susceptible to the organophosphates dichlorvos, malathion, chlorpyrifos-methyl, and pirimiphos-methyl), and to the pyrethroids deltamethrin and cypermethrin than the laboratory strain based on discriminating-dose tests with treated filter papers (Kljajic and Peric 2006). Four field strains of *O. surinamensis* collected from stored barley on Minnesota farms were less susceptible to chlorpyrifos-methyl compared with a laboratory strain, even before the insecticide was registered for use on this commodity, indicating natural tolerance (Subramanyam et al. 1989). The wild strains of *O. surinamensis* in Australia showed low resistance levels (<10-fold) to chlorpyrifos-methyl in treated filter paper assays (Attia and Frecker 1984). Strains of *R. dominica* from Brazil were found to be 2 to 874 more resistant to deltamethrin than a susceptible laboratory strain (Lorini and Galley 1999). Guedes et al. (1996) detected resistance to chlorpyrifos-methyl in *R. dominica* strains collected from Brazil and Kansas, USA, with resistance ratios at the median lethal concentration (LC_{50}) ranging from 5.6 to 167.9. Perez-Mendoza (1999) found a low level of resistance (1.2 to 1.8-fold) to deltamethrin in 11 field strains of the maize weevil, *Sitophilus zeamais* Motschulsky, collected from nine states in Mexico.

In Australia, resistance to cyfluthrin has been reported in *O. surinamensis* (Collins and Wilson 1987) and *T. castaneum* (Collins 1990) using filter paper and treated grain assays. Resistance to cyfluthrin is also reported in other insect species such as the housefly, *Musca domestica* L. (Kaufman et al. 2001); German cockroach, *Blattella germanica* (L.) (Cochran 1989, Chai and Lee 2010); beet armyworm, *Spodoptera exigua* (Hübner) (Aldosari et al. 1996) and lesser meal worm, *Alphitobius diaperinus* (Panzer) (Hamm et al. 2006).

In the present study, *R. dominica* showed reduced susceptibility to chlorpyrifos-methyl plus deltamethrin. Some previous studies have reported the field strains of *R. dominica* to be resistant to chlorpyrifos-methyl (Zettler and Cuperus 1990, Beeman and Wright 1990, Guedes et al. 1996) and to deltamethrin (Lorini and Galley 1999). Subramanyam et al. (2007) reported chlorpyrifos-methyl at 3.0 mg (AI)/kg of grain to be effective against *T. castaneum* and *O. surinamensis* on stored wheat but not against *R. dominica*.

The results on insect susceptibility to the tested insecticides on concrete surfaces is at variance from that observed with the same insecticides on stored grain. For example, on stored rice and wheat chlorpyrifos-methyl plus deltamethrin was effective against *Lepinotus reticulatus* Enderlein, *Liposcelis entomophila* (Enderlein), *Liposcelis bostrychophila* Badonnel, and

Liposcelis paeta Pearman (Athanassiou et al. 2009). Subramanyam et al. (2012) also reported insecticide to be effective at the labeled rate against *R. dominica*, *S. oryzae*, *T. castaneum*, and *P. interpunctella* on wheat and *R. dominica* and *S. oryzae* on short-grain and long-grain rices. The reduced susceptibility of *T. castaneum* and *O. surinamensis* strains to chlorpyrifos-methyl plus deltamethrin and β-cyfluthrin on concrete surfaces as opposed to grain could be due to absorption or loss of the sprayed solution into the porous concrete. The concrete surface is also alkaline (pH ~10.5) and may have hydrolyzed the insecticide (White and Leesch 1996). The persistence of cyfluthrin can be increased by sealing concrete with various commercial sealants (Arthur 1994). An additional factor reducing the efficacy of insecticides on concrete may be uneven spray deposition during application, leading to areas with little or no insecticide deposit. Insects seeking such areas may not receive a lethal dose of the insecticide.

On grain both contact and ingestion toxicity is important whereas on concrete there is only contact toxicity. Generally, on grain insects are exposed to 7 d or more, whereas on concrete surfaces the maximum exposure time was 24 h. Such short exposures may have been sublethal (Guedes et al. 2011) and allowed insect recovery when placed on diets. Arthur (1999d) reported that exposure of *R. dominica* adults for 24 h or less on wheat treated with cyfluthrin emulsifiable concentrate at 1, 2, and 4 mg (AI)/kg gave less than 90% mortality.

The poor effectiveness of β-cyfluthrin against *T. castaneum* field strains at 0.02 to 0.08 g (AI)/m^2 rate in this study is in contrast to excellent control shown by cyfluthrin wettable powder against a laboratory strain of *T. castaneum* at 0.04 g (AI)/m^2 (Arthur 1998a). The wettable powder formulation gave 90% mortality of *T. castaneum* adults when exposed for 0.5 to 4.0 h on deposits aged 8 to 24 wk (Arthur 1998a). Similar data on *O. surinamensis* with a wettable powder formulation are not available for comparisons. β-cyfluthrin, an enriched isomeric form of the two biologically active diastereoisomeric pairs of isomers of cyfluthrin, should perform better or as well as the wettable powder formulation. For example, β-cyfluthrin showed high short-term efficacy with time to 95% mortality (LT_{95}) of 12 to15 h against stored-product psocids, *L. bostrychophila* and *L. entomophila* on concrete when applied at a much lower rate of 0.002 g (AI)/m^2 (Guedes et al. 2008). Kaufman and Rutz (2002) reported that the wettable powder formulation of cyfluthrin was more toxic than suspension concentrate formulation applied on painted and unpainted plywood panels against *M. domestica* collected from dairies in the State of New York.

Except for *R. dominica* strains exposed to β-cyfluthrin, in our study percentage mortality of *T. castaneum* and *O. surinamensis* strains was generally lower than knockdown indicating recovery when placed on diets. Recovery of insects on food after a brief insecticide exposure may be due to adsorption of insecticide from the insect's integument by the food particles, or an increase in insect's ability to detoxify the insecticide after removal from treated substrates (Arthur 2000). The time for 90% mortality of beetles (LT_{90}) placed on food for one week after exposure to 0.02 g(AI)/m^2 of cyfluthrin wettable powder for 0.5 to 2.0 h was 195 min whereas LT_{90} for those without food was 19 min (Arthur 1998b). The presence of flour on methoprene-treated concrete surfaces reduced the efficacy of methoprene against *T. castaneum* late-instar larvae (10 to 12 d old from hatching) (Wijayaratne et al. 2012). Therefore, sanitation of empty storage surfaces is very important to improve effectiveness of residual insecticides. The fact that there is recovery indicates that the insecticides did not exhibit any delayed effects. Delayed mortality effects have been reported in *R. dominica* (Boina et al. 2012, Getchell and Subramanyam 2008) but not in *S. oryzae* after short exposures to spinosad-treated wheat (Getchell and Subramanyam 2008).

Except for *R. dominica* strains exposed to β-cyfluthrin and *T. castaneum* to chlorpyrifos-methyl plus deltamethrin at constant conditions, progeny was observed among strains in all other insecticide treatments. Progeny production was generally lower for adults exposed to insecticides when compared to those in the control treatment, because of fewer surviving adults. Wide variation was also noticed in progeny production among strains of a species on untreated or treated surfaces. It is unclear whether differences in progeny production among strains is related to insecticide resistance or to natural biological differences among strains. Additional dose-response or time-response tests are needed over several generations to confirm resistance since it is an inherited trait.

In the present study, β-cyfluthrin was found to be more effective at the room temperature of ~25°C than at 28°C against *T. castaneum* and *O. surinamensis*. These strains and those of *R. dominica* were more susceptible at 28°C than at 25°C when exposed to chlorpyrifos-methyl plus deltamethrin. Cyfluthrin toxicity was negatively correlated with temperatures of 20, 25, 30, and 35^{o}C when tested against *T. castaneum* (Arthur 1999c), *T. confusum*, the larger grain borer, *Prostephanus truncatus* (Horn); *P. interpunctella*; and the almond moth, *Cadra cautella* (Walker), but was neutral for *R. dominica* (Subramanyam and Cutkomp 1987).

Organophosphate insecticides such as chlorpyrifos-methyl are more toxic at higher than lower temperatures against *O. surinamensis* (Barson 1983), *T. castaneum*, and *S. granarius* (Tyler and Binns 1982). O'Donnell (1980) found positive correlation between temperature and effectiveness of organophosphate insecticides like malathion, pirimiphos-methyl, and fenitrothion in *T. confusum*. Deltamethrin displayed negative temperature coefficient (more toxic at cooler temperatures) against the cabbage looper, *Trichoplusia ni* (Hubner), and boll weevil, *Anthonomus grandis grandis* Boheman, but exhibited either neutral or positive temperature coefficient (more toxic at warmer temperatures) against the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and tobacco budworm, *Heliothis virescens* (F.) (Sparks et al.1982, 1983). An overall negative temperature coefficient was observed for deltamethrin emulsifiable concentrate formulation when tested against nymphs of the grasshopper *Melanoplus* spp. at 15.6 to 37.8°C with a slight positive temperature coefficient at 21.1 to 26.7°C (Hinks 1985). However, a neutral or positive correlation was reported with a flowable formulation of deltamethrin when tested at 15 to 31°C against *Melanoplus* spp. (Johnson 1990). We hypothesize that deltamethrin in the combination product may have exhibited either neutral or positive temperature coefficient.

Our results show that β-cyfluthrin is an ideal insecticide to use in clean, empty bin floors prior to storing wheat only to control *R. dominica* adults but not *T. castaneum* and *O. surinamensis* strains. The reduced susceptibility of field and laboratory strains of the latter two species may be due to an inherent formulation deficiency or resistance, since four times the labeled rate failed to provide complete control and progeny suppression. Chlorpyrifos-methyl plus deltamethrin was only partially effective against strains of all three species. There is documented evidence of resistance in field strains of these three species to one or both active ingredients. This is the first report that characterized susceptibility, or lack thereof, of field strains of three insect species from Kansas to two approved insecticides used for empty-bin treatments. According to surveys of wheat stored on-farm and elevators in Kansas, the most common insect species associated with stored wheat are *R. dominica*, *S.oryzae* and *T. castaneum*. In addition, *Oryzaephilus* spp. and *Cryptolestes* spp. are also found in stored grain in Kansas (Reed et al. 1991, 2003). Based on our results, no single insecticide can be recommended to provide adequate control of all species tested. More work is needed on the mechanism of detoxification of different chemicals by different species to understand why some chemicals are

effective against some species and not against others. Evaluation of other recommended empty-bin insecticides with the field strains is also needed to identify a broad-spectrum insecticide that is effective against species commonly found in empty bins.

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Figure 1.1 Mean ± SE (*n* = 3) observed and predicted adult knockdown and mortality of laboratory strains of three insect species as a function of time when exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces.

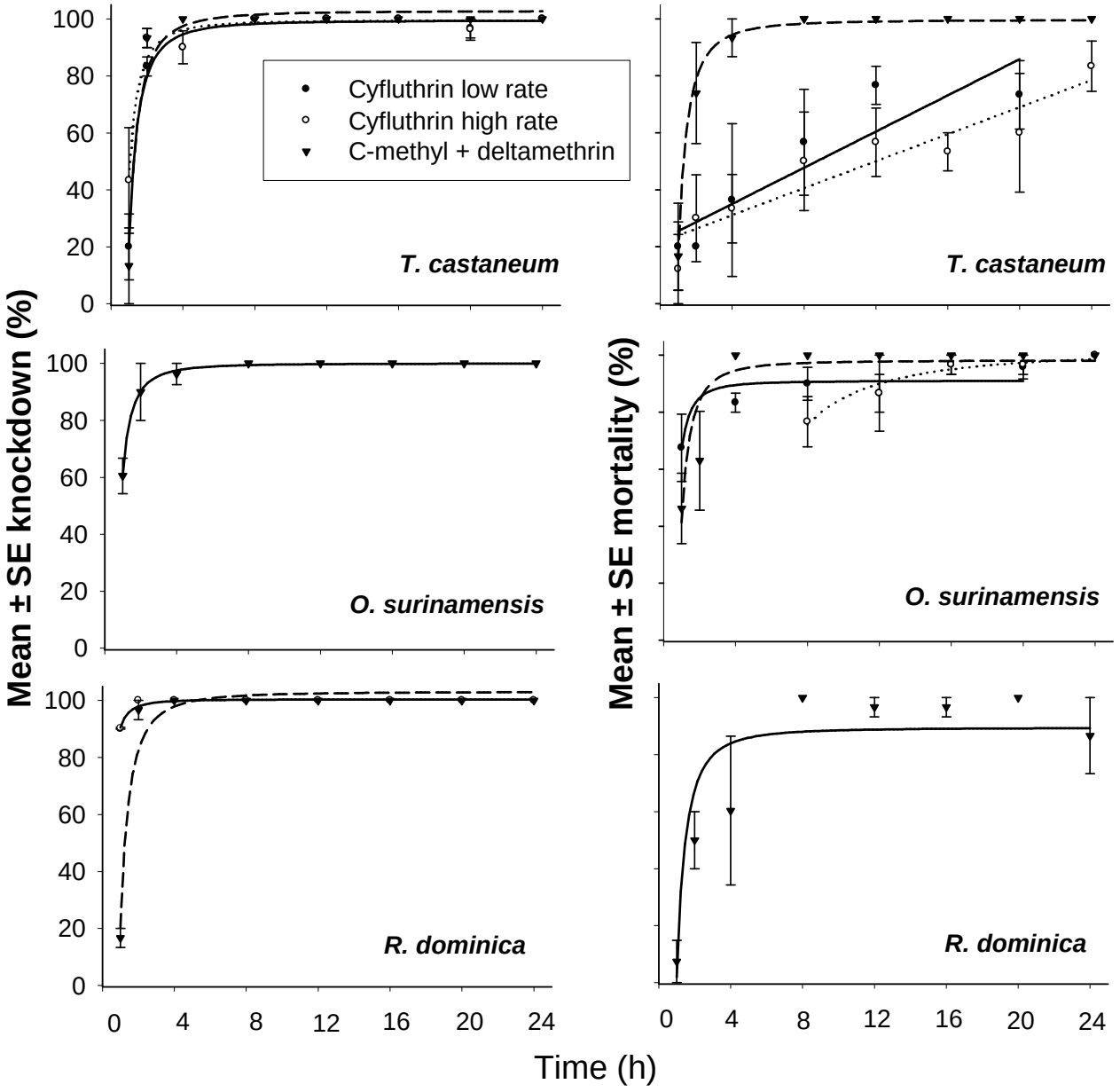
Mean ± SE knockdown (%)
Mean ± SE mortality (%)
Cyfluthrin low rate
Cyfluthrin high rate
C-methyl + deltamethrin
T. castaneum
T. castaneum
O. surinamensis
O. surinamensis
R. dominica
R. dominica
0
20
40
60
80
100
0
4
8
12
16
20
24
Time (h)

Figure 1.2 Mean ± SE (*n* = 5) knockdown and mortality of adults of laboratory and field strains of three insect species exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces at room conditions (25.4°C and 17.2% RH) For each species and response, means for a strain followed by an asterisk (*) is significantly different from the corresponding laboratory strain (*P* < 0.05; by Dunnett's test).

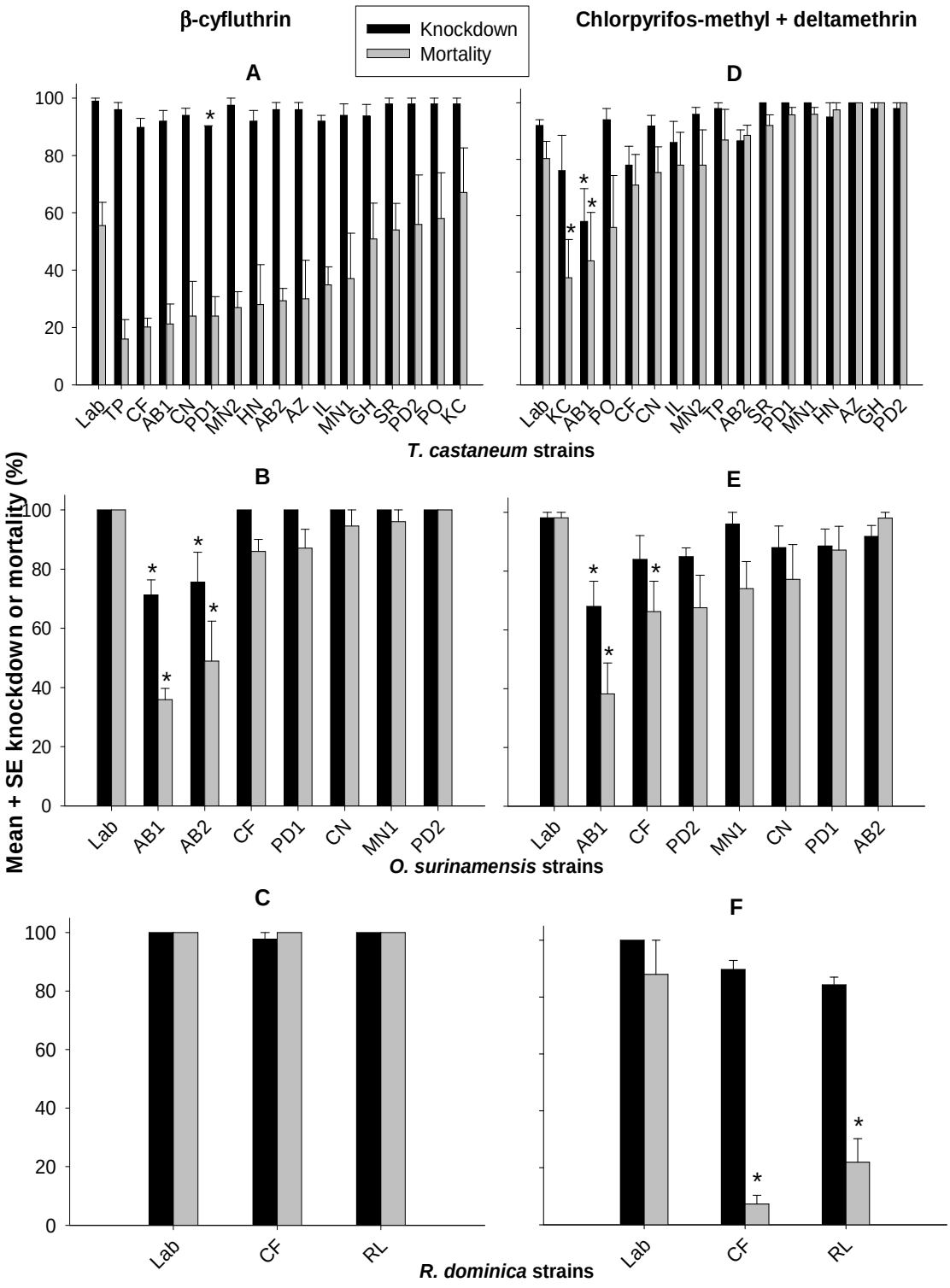
β-cyfluthrin
Knockdown
Mortality
Chlorpyrifos-methyl + deltamethrin
A
D
100
80
60
40
20
0
Lab TP CF AB1 CN PD1 MN2 HN AB2 AZ IL MN1 GH SR PD2 PO KC
Lab KC AB1 PO CF CN IL MN2 TP AB2 SR PD1 MN1 HN AZ GH PD2
T. castaneum strains
Mean + SE knockdown or mortality (%)
B
E
Lab AB1 AB2 CF PD1 CN MN1 PD2
Lab AB1 CF PD2 MN1 CN PD1 AB2
O. surinamensis strains
C
F
Lab CF RL
Lab CF RL
R. dominica strains

Figure 1.3 Mean ± SE (*n* = 5) knockdown and mortality of adults of laboratory and field strains of three insect species exposed to β-cyfluthrin and chlorpyrifos-methyl plus deltamethrin treated concrete surfaces at constant conditions (28°C and 65% RH). For each species and response, means for a strain followed by an asterisk (*) is significantly different from the corresponding laboratory strain (*P* < 0.05; by Dunnett's test).

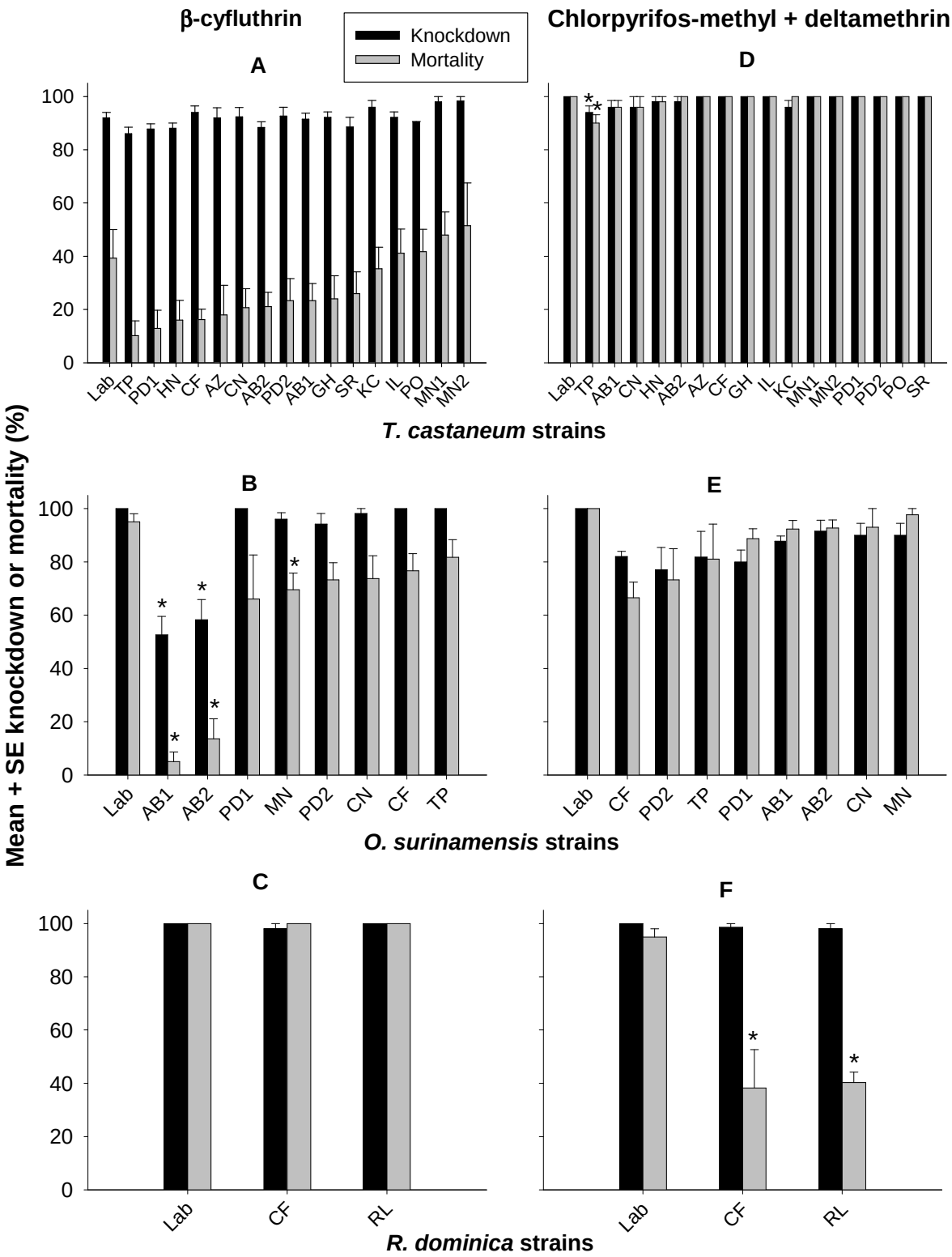
β-cyfluthrin
Knockdown
Mortality
Chlorpyrifos-methyl + deltamethrin
A
D
B
E
C
F
Mean + SE knockdown or mortality (%)
T. castaneum strains
O. surinamensis strains
R. dominica strains
Lab
TP
PD1
HN
CF
AZ
CN
AB2
PD2
AB1
GH
SR
KC
IL
PO
MN1
MN2
AB1
AB2
MN
CF
RL

Table 1.1 List of approved insecticides for stored-grain insect management

Product	Active ingredient	Rate (mg[AI]/kg)	Site treated	Commodity
Storcide II	Chlorpyrifos-methyl + deltamethrin	3 + 0.5	Empty bins, warehouses, grain	Wheat, barley, rice, oats, sorghum
Tempo SC Ultra	β-cyfluthrin	0.01 & 0.02 g/m^2	Empty bins	
Actellic 5E	Pirimiphos-methyl	6 - 8	Stored grain	Corn, sorghum
K-Othrine SC, Centynal	Deltamethrin	0.5	Empty bins, warehouses, grain	Wheat, barley, rice, oats, sorghum, corn, rye
Evercide	Esfenvalerate			
Diasource, Dryacide, Protect-It	Diatomaceous earth (silicon dioxide)	500 - 1000	Empty bins, stored grain	Wheat, barley, rice, oats, sorghum, corn, peas
Diacon-IGR	S-methoprene	1, 2.5 & 5	Empty bins, stored grain	All stored grains, spices, seeds
Spinosad	Spinosyns A + D	1	Grain	All stored grains, including wheat

Table 1.2 Sites and dates of collection of adult *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains from Kansas farms in 2011

Species	Strain ID	County, State	Location	Commodity	Collection dates
T. castaneum	AB1	Dickinson, KS	Abilene	Wheat	Aug. 3, 10
	AB2	Dickinson, KS	Abilene	Wheat	Aug. 24, Sept. 12, Oct. 4
	AZ	Maricopa, AZ[a]	____[b]	Flour mill	2009
	CF	Washington, KS	Clifton	Wheat	Aug. 3, 24
	CN	McPherson, KS	Canton	Wheat	Sept. 20
	GH	Russell, KS	Gorham	Wheat	Oct. 4
	HN	Stafford, KS[a]	____	Flour mill	2001
	IL	Cook, IL[c]	Bridgeview	Rice facility	2011
	KC	Jackson, MO[a]	____	Flour mill	2005
	MN1	Ottawa, KS	Minneapolis	Wheat	Aug. 10
	MN2	Ottawa, KS	Minneapolis	Wheat	Aug. 10
	PD1	Russell, KS	Paradise	Wheat	Aug. 18, 30, Sept. 13
	PD2	Russell, KS	Paradise	Corn	Aug. 18, 30, Sept. 13
	PO	KS[a]	____	Flour mill	2001
	SR	Dickinson, KS[a]	____	Flour mill	2001
	TP	Mitchell, KS	Tipton	Wheat	Aug. 18
O. surinamensis	AB1	Dickinson, KS	Abilene	Wheat	Aug. 3, 10
	AB2	Dickinson, KS	Abilene	Wheat	Aug. 24, Sept. 12, Oct. 4
	CF	Washington, KS	Clifton	Wheat	Aug. 3, 24
	CN	McPherson, KS	Canton	Wheat	Sept. 20
	MN1	Ottawa, KS	Minneapolis	Wheat	Aug. 10
	PD1	Russell, KS	Paradise	Wheat	Aug. 18, 30, Sept. 13

	PD2	Russell, KS	Paradise	Corn	Aug. 18, 30, Sept. 13
	TP	Mitchell, KS	Tipton	Wheat	Aug. 18
R. dominica	CF	Washington, KS	Clifton	Wheat	Aug. 3, 24
	RL	Riley, KS[a]	____	Flour mill	2007

[a]These strains were collected prior to 2011 and were provided by Dr. James Campbell, USDA-ARS, Center for Grain and Animal Health Research, Manhattan, KS, USA.

[b]Exact city name is not disclosed at the request of the mill manager.

[c]Strain collected by one of the authors (Bhadriraju Subramanyam) during a visit to a rice-processing facility in 2011.

Table 1.3 Parameter estimates for regression models fit to knockdown (KD) and mortality (M) data of four insect species exposed to insecticide deposits on concrete

Species	Insecticide	Response	*n*	Mean ± SE for parameters		r^2
				a	*b*	
T. castaneum	β-cyfluthrin low rate	KD	8	99.5 ± 1.1	-78.9 ± 3.0	0.99
		M[a]	6	22.3 ± 7.8	3.2 ± 0.8	0.81
	β-cyfluthrin high rate	KD	8	99.6 ± 1.7	-54.7 ± 4.6	0.96
		M[a]	8	21.6 ± 5.1	2.4 ± 0.4	0.87
	Chlorpyrifos-methyl	KD	8	102.8 ± 2.2	-86.4 ± 6.1	0.97
	plus deltamethrin	M	8	99.6 ± 0.9	-84.1 ± 2.4	0.99
O. surinamensis	β-cyfluthrin low rate	KD		—[b]	—	
		M	6	91.0 ± 2.4	-23.9 ± 5.9	0.80
	β-cyfluthrin high rate	KD		—	—	
		M	5	101.5 ± 1.7	-1655. 8 ± 211.6	0.95
	Chlorpyrifos-methyl	KD	8	100.0 ± 0.2	-39.6 ± 0.6	0.99
	plus deltamethrin	M	8	98.2 ± 3.7	-56.8 ± 10.1	0.84
R. dominica	β-cyfluthrin low rate	KD	8	100.4 ± 0.4	-9.9 ± 1.0	0.94
		M		—	—	
	β-cyfluthrin high rate	KD	8	100.4 ± 0.4	-9.6 ± 1.0	0.94
		M		—	—	
	Chlorpyrifos-methyl	KD	8	103.1 ± 2.6	-82.8 ± 7.1	0.96
	plus deltamethrin	M	8	89.4 ± 5.8	-87.6 ± 15.9	0.83

[a] Linear equation $y = a + bx$ was fit to the data; all other responses were fit to the non-linear equation $y = a + b/x^2$

[b] Knockdown or mortality at all observation times was 100%.

Table 1.4 Comparison of knockdown (KD) or mortality (M) responses between insecticides and rates tested by species

Species	Response	Insecticides compared	*F*-value	df	*P*-value
T. castaneum	KD	β-cyfluthrin low rate vs β-cyfluthrin high rate	12.16	2, 12	0.001*
		β-cyfluthrin low rate vs Chlorpyrifos -methyl plus deltamethrin	1.05	2, 12	0.380
		β-cyfluthrin high rate vs Chlorpyrifos -methyl plus deltamethrin	8.81	2, 12	0.004*
	M	β-cyfluthrin low rate vs β-cyfluthrin high rate	1.52	2, 10	0.266
O. surinamensis	M	β-cyfluthrin low rate vs β-cyfluthrin high rate	9.18	2, 7	0.011*
		β-cyfluthrin low rate vs Chlorpyrifos -methyl plus deltamethrin	3.59	2, 10	0.067
		β-Cyfluthrin high rate vs Chlorpyrifos -methyl plus deltamethrin	3.77	2, 9	0.065
R. dominica	KD	β-cyfluthrin low rate vs β-cyfluthrin high rate	0.03	2, 12	0.971
		β-cyfluthrin low rate vs Chlorpyrifos -methyl plus deltamethrin	59.83	2, 12	<0.001*
		β-cyfluthrin high rate vs Chlorpyrifos -methyl plus deltamethrin	60.39	2, 12	<0.001*

* Significant ($P < 0.05$).

Some insecticide combinations were not compared because of 100% knockdown or mortality at all observation times.

Table 1.5 Comparison of knockdown (KD) or mortality (M) responses between species by insecticides and rate

Insecticide	Response	Species compared	*F*-value	df	*P*-value
β-cyfluthrin low rate	KD	*T. castaneum* vs *R. dominica*	307.95	2, 12	<0.0001*
β-cyfluthrin high rate	KD	*T. castaneum* vs *R. dominica*	60.44	2, 12	<0.0001*
Chlorpyrifos -methyl	KD	*T. castaneum* vs *R. dominica*	0.11	2, 12	0.895
plus deltamethrin		*T. castaneum* vs *O. surinamensis*	31.90	2, 12	<0.001*
		R. dominica vs *O.surinamensis*	19.87	2, 12	0.0001*
	M	*T. castaneum* vs *R. dominica*	2.15	2, 12	0.159
		T. castaneum vs *O. surinamensis*	3.87	2, 12	0.050
		R. dominica vs *O. surinamensis*	3.91	2, 12	0.049*

* Significant ($P < 0.05$).

Table 1.6 Adult progeny production of *T. castaneum* laboratory strain exposed for various time periods to insecticide deposits on concrete[a]

Treatment	Mean ± SE progeny exposed for (h)							
	1	2	4	8	12	16	20	24
Control	571.3 ± 48.8	482.3 ± 90.5	337.3 ± 89.1	305.7 ± 28.4	229.7 ± 37.4	218.3 ± 10.8	355.7 ± 6.0	242.0 ± 17.7
Cyfluthrin low rate	218.3 ± 82.9	165.3 ± 16.8	103.3 ± 58.9	46.7 ± 20.1	12.7 ± 7.8	8.7 ± 4.2	28.7 ± 21.2	57.3 ± 20.3
Cyfluthrin high rate	189.0 ± 39.4	95.0 ± 47.6	97.7 ± 85.5	65.0 ± 40.1	10.3 ± 10.3	36.3 ± 22.7	47.3 ± 35.6	27.3 ± 27.3
C-methyl + deltamethrin	441.3 ± 65.8	64.7 ± 64.7	5.0 ± 5.0	0	0	0	0	0

[a] Parental adults after exposure for specific time periods on insecticide-treated concrete were placed on 30 g of wheat flour at 28°C and 65% RH for 1 wk to assess mortality, after which all adults were discarded and flour incubated for 35 d to count adult progeny produced.

Each mean is based on $n = 3$.

Table 1.7 Adult progeny production of *O. surinamensis* laboratory strain exposed for various time periods to insecticide deposits on concrete[a]

Treatment	Mean ± SE progeny exposed for (h)							
	1	2	4	8	12	16	20	24
Control	195.3 ± 53.7	226.0 ± 4.7	137.3 ± 29.7	233.0 ± 46.1	177.3 ± 6.4	188.7 ± 19.9	179.0 ± 117.2	165.0 ± 7.2
Cyfluthrin low rate	11.0 ± 1.7	18.0 ± 17.5	21.3 ± 18.3	10.7 ± 5.3	17.7 ± 16.7	0.7 ± 0.7	1.7 ± 1.7	0.7 ± 0.7
Cyfluthrin high rate	15.3 ± 15.3	38.0 ± 22.9	16.0 ± 8.0	28.7 ± 16.2	6.3 ± 3.3	1.3 ± 0.7	0	0
C-methyl + deltamethrin	124.3 ± 13.4	52.3 ± 51.8	0.3 ± 0.3	0.3 ± 0.3	0	0	0	0.3 ± 0.3

[a] Parental adults after exposure for specific time periods on insecticide-treated concrete were placed on 30 g of rolled oats at 28°C and 65% RH for 1 wk to assess mortality, after which all adults were discarded and oats incubated for 35 d to count adult progeny produced.

Each mean is based on $n = 3$.

Table 1.8 Adult progeny production of *R. dominica* laboratory strain exposed for various time periods to insecticide deposits on concrete[a]

Treatment	Mean ± SE progeny exposed for (h)							
	1	2	4	8	12	16	20	24
Control	43.7 ± 23.0	90.7 ± 26.8	59.7 ± 14.9	52.0 ± 13.6	64.7 ± 21.5	98.7 ± 43.1	174.7 ± 19.2	153.0 ± 62.6
Cyfluthrin low rate	0	0.3 ± 0.3	0	0	0.7 ± 0.7	0	0	0
Cyfluthrin high rate	0	0	0	0	0	0	0	0
C-methyl + deltamethrin	181.3 ± 67.5	107.0 ± 25.9	102.0 ± 86.2	0	0.3 ± 0.3	15.3 ± 15.3	0	11.0 ± 11.0

[a] Parental adults after exposure for specific time periods on insecticide-treated concrete were placed on 30 g of whole wheat at 28°C and 65% RH for 1 wk to assess mortality, after which all adults were discarded and wheat incubated for 35 d to count adult progeny produced.

Each mean is based on $n = 3$.

Table 1.9 Adult progeny production of field strains of three insect species exposed to insecticide-treated concrete surfaces at room conditions[a,b]

Insect species	Strain	Progeny (Mean ± SE)		
		Control	β-cyfluthrin high rate	Chlorpyrifos -methyl plus deltamethrin
T. castaneum	Lab.	362.1 ± 19.7	80.4 ± 27.7	40.0 ± 20.1
	AB1	470.2 ± 14.5	258.2 ± 27.6	146.8 ± 56.2
	AB2	435.2 ± 12.2	207.0 ± 40.7	22.6 ± 12.8
	CF	449.0 ± 35.3	276.0 ± 20.7	63.8 ± 25.8
	CN	473.0 ± 10.2	221.8 ± 41.8	19.4 ± 9.3
	GH	306.8 ± 35.0	107.2 ± 40.4	0
	HN	463.2 ± 29.7	154.2 ± 23.8	18.2 ± 13.7
	MN1	395.2 ± 11.7	145.0 ± 42.6	6.0 ± 3.7
	MN2	425.0 ± 22.3	147.0 ± 13.8	30.0 ± 11.3
	PD1	375.0 ± 70.4	217.0 ± 24.5	0.4 ± 0.4
	PD2	441.7 ± 28.6	90.2 ± 44.4	0
	PO	397.0 ± 3.4	77.4 ± 36.9	78.6 ± 33.0
	SR	541.0 ± 14.5	248.2 ± 54.6	43.8 ± 23.8
	TP	435.0 ± 17.2	275.0 ± 25.6	40.2 ± 36.3
	KC	414.0 ± 51.7	112.0 ± 59.4	141.6 ± 47.9
	AZ	442.6 ± 24.3	194.2 ± 49.6	0
	IL	446.4 ± 22.0	214.6 ± 18.5	60.8 ± 33.9
O. surinamensis	Lab.	168.7 ± 21.6	0.6 ± 0.4	9.8 ± 8.6

	AB1	11.0 ± 6.3	22.8 ± 4.7	0.8 ± 0.6
	AB2	16.6 ± 4.4	33.8 ± 14.6	0
	CN	7.0 ± 3.4	0	4.4 ± 2.8
	CF	37.6 ± 10.0	6.2 ± 4.3	5.4 ± 3.4
	MN1	74.2 ± 11.2	4.4 ± 4.4	19.8 ± 8.1
	PD1	74.0 ± 14.6	0.2 ± 0.2	15.2 ± 10.5
	PD2	37.0 ± 8.5	0	11.8 ± 5.1
R. dominica	Lab.	94.4 ± 26.8	0.2 ± 0.2	16.2 ± 16.2
	CF	68.6 ± 15.1	0	83.0 ± 25.5
	RL	115.4 ± 21.3	0.4 ± 0.4	73.6 ± 20.4

[a]Mean ± SE temperature and relative humidity of the room during tests were 25.4°C and 17.2%, respectively.

[b]Each mean is based on $n = 5$.

Table 1.10 Adult progeny production of field strains of three insect species exposed to insecticide-treated concrete surfaces at constant conditions[a,b]

Insect species	Strain	Mean ± SE Progeny	
		β-cyfluthrin high rate	Chlorpyrifos-methyl plus deltamethrin
T. castaneum	Lab.	5.2 ± 3.8	0
	AB1	28.2 ± 16.3	0
	AB2	35.2 ± 9.3	0
	CF	124.2 ± 29.4	0
	CN	130.2 ± 27	0
	GH	125.8 ± 41	0
	HN	187.0 ± 24.4	3.0 ± 3.0
	MN1	19.2 ± 7.8	0
	MN2	47.4 ± 20.2	0
	PD1	131.6 ± 32.6	0
	PD2	80.8 ± 22.3	0
	PO	73.2 ± 42.0	0
	SR	216.2 ± 50.4	0
	TP	158.2 ± 35.1	0
	KC	82.2 ± 30.7	0
	AZ	158.6 ± 65.8	0
	IL	160.6 ± 35.6	0
O. surinamensis	Lab.	1.2 ± 1.2	0

	AB1	32.8 ± 7.8	0.2 ± 0.2
	AB2	47.2 ± 7.2	0.2 ± 0.2
	CF	6.8 ± 4.0	4.0 ± 2.3
	CN	5.4 ± 4.9	0
	MN	12.4 ± 5.6	0
	PD1	0.2 ± 0.2	0
	PD2	5.4 ± 5.2	0.2 ± 0.2
	TP	0.4 ± 0.2	0
R. dominica	Lab.	0	0
	CF	0.8 ± 0.8	17.2 ± 3.6
	RL	0	17.6 ± 4.4

[a]The mean ± SE progeny production of *T. castaneum*, *R. dominica*, and *O. surinamensis* laboratory strains in control treatment was 152.2 ± 27.2, 64.2 ± 17.1, and 142.6 ± 35.1, respectively.

[b]Each mean is based on $n = 5$.

Table 1.11 Knockdown and mortality of laboratory and select least susceptible field strains of *T. castaneum and O. surinamensis* exposed to concrete surfaces treated with one to four times the high labeled rate of β-cyfluthrin[a,b]

Strain	Mean ± SE knockdown (%) at β-cyfluthrin rate (g[AI]/m^2) of:				Mean ± SE mortality (%) at β-cyfluthrin rate (g[AI]/m^2) of:			
	0.02	0.04	0.06	0.08	0.02	0.04	0.06	0.08
T. castaneum								
Lab.	96.0 ± 2.4[c]	100	98.0 ± 2.0	98.0 ± 2.0	72.0 ± 10.7[d]	74.0 ± 5.1	84.0 ± 10.3	90.0 ± 7.7
CF	98.0 ± 2.0[c]	100	100	100	54.5 ± 12.6[d]	61.0 ± 10.5	55.1 ± 17.0	77.6 ± 12.4
PD1	98.0 ± 2.0[c]	100	100	100	66.0 ± 12.5[d]	90.0 ± 7.7	72.0 ± 7.3	78.0 ± 4.9
TP	96.0 ± 2.4[c]	100	100	100	54.0 ± 9.3b	82.2 ± 5.0a	74.7 ± 7.1ab	90.0 ± 3.2a
O. surinamensis								
Lab.	100	100	100	100	100	100	100	100
AB1	71.3 ± 3.9b	93.9 ± 2.5a	85.8 ± 6.8ab	92.0 ± 3.7a	58.7 ± 4.9[e]	76.9 ± 9.3	57.1 ± 10.7	70.9 ± 9.9
AB2	80.0 ± 8.4b	98.0 ± 2.0a	100.0a	96.0 ± 2.4a	36.0 ± 10.3[e]	71.4 ± 7.2	60.0 ± 10.5	70.2 ± 6.5

[a]Each mean is based on $n = 5$.

[b]For each strain and response (knockdown or mortality), means among rates followed by different letters are significantly different ($P < 0.05$; by least squares means test).

[c]For each *T. castaneum* strain, knockdown among rates was not significant (*F*, range among strains = 0.76 - 2.67; df = 3, 16; *P*, range = 0.083 - 0.532; one-way ANOVA).

[d]For *T. castaneum* Lab., CF, or PD1 strain, mortality among rates was not significant (*F*, range among strains = 0.91 - 2.11; df = 3, 16; *P*, range = 0.139 - 0.459; one-way ANOVA).

[e]For *O. surinamensis* AB1 or AB2 strain, mortality among rates was not significant (*F*, range between strains = 1.22 - 2.62; df = 3, 16; *P*, range = 0.087 - 0.336; one-way ANOVA).

Table 1.12 Progeny of laboratory and least susceptible *T. castaneum and O. surinamensis* strains exposed to concrete surfaces treated with one to four times the high labeled rate of β-cyfluthrin

Strain	Mean ± SE[a] adult progeny produced at β-cyfluthrin rate (g[AI]/m^2) of:				
	0	0.02	0.04	0.06	0.08
T. castaneum					
Lab.	317.6 ± 61.4	18.8 ± 8.8	38.4 ± 14.0	3.0 ± 2.8	6.2 ± 6.2
CF	287.0 ± 8.1	40.6 ± 23.8	79.4 ± 27.0	72.6 ± 44.1	39.4 ± 28.2
PD1	337.3 ± 20.3	37.4 ± 26.1	4.8 ± 2.9	50.2 ± 6.0	54.0 ± 24.6
TP	357.4 ± 42.4	89.6 ± 35.2	30.8 ± 18.4	26.2 ± 16.0	3.6 ± 2.4
O. surinamensis					
Lab.	100.4 ± 26.9	1.0 ± 0.8	0	0	0
AB1	71.8 ± 24.4	18.4 ± 4.1	6.8 ± 2.9	8.2 ± 3.2	4.2 ± 2.0
AB2	86.0 ± 27.1	24.6 ± 6.6	6.2 ± 6.0	5.4 ± 2.2	6.2 ± 4.2

[a]Each mean is based on $n = 5$; at each n, 10 insects were exposed

Chapter 2 - Variation in susceptibility of laboratory and field strains of three stored-grain insect species to spinosad and chlorpyrifos-methyl plus deltamethrin on stored wheat

Abstract

Spinosad and chlorpyrifos-methyl plus deltamethrin efficacy at labeled rates on hard red winter wheat were evaluated against 11 strains of the red flour beetle, *Tribolium castaneum* (Herbst); six strains of the sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); and two strains of the lesser grain borer, *Rhyzopertha dominica* (F.), collected mostly from farm-stored grain in Kansas. Adults (25) of each species were exposed to wheat treated with spinosad at 1 mg(AI)/kg or chlorpyrifos-methyl plus deltamethrin at 3 plus 0.5 mg(AI)/kg. Adult mortality was assessed after 7 and 14 d and progeny production after 42 d. Spinosad did not provide complete mortality or progeny suppression of *T. castaneum* and *O. surinamensis* field strains, but was effective against *R. dominica* strains. Chlorpyrifos-methyl plus deltamethrin produced complete mortality and progeny suppression of field strains all three species. The two least susceptible *T. castaneum* and *O. surinamensis* strains and the two *R. dominica* strains were selected for dose-response tests only with spinosad. The LD_{99} values for *T. castaneum* and *R. dominica* field strains were similar to that of the corresponding laboratory strains. Corresponding values for the two *O. surinamensis* field strains were significantly greater (~6 times) than the laboratory strain. The effective dose for progeny reduction (ED_{99}) of only one *R. dominica* field strain was significantly greater (~2 times) than the laboratory strain. The baseline susceptibility data of field strains of three insect species to spinosad will be useful for monitoring resistance development when this product is commercially released as a grain protectant.

KEY WORDS stored-grain insects, spinosad, chlorpyrifos-methyl plus deltamethrin, field strains, mortality, progeny suppression

Introduction

The application of an insecticide to newly-harvested grain as it is loaded into a bin is an important preventive integrated pest management approach. Insecticides applied directly to grain are called grain protectants. Spinosad was approved as a grain protectant by the United States Environmental Protection Agency in 2005 for use on barley, corn, millets, oats, rice, sorghum, and wheat (Hertlein et al. 2011), and commercial release of formulations for grain treatment is expected soon. Chlorpyrifos-methyl plus deltamethrin was registered in 2004 for use on barley, oats, rice, sorghum, and wheat as well as empty bins receiving these grains. This combination product replaced chlorpyrifos-methyl after its tolerances were revoked on December 31, 2004.

Spinosad at the labeled rate of 1 mg (AI)/kg is effective against a broad range of stored-grain insects (Fang et al. 2002a, Subramanyam et al. 2007, Hertlein et al. 2011, Subramanyam et al. 2012), on barley, corn, sorghum, and wheat (Chintzoglou et al. 2008, Getchell and Subramanyam 2008, Athanassiou et al. 2010, Kavallieratos et al. 2010, Vayias et al. 2010). Previous studies have shown the lesser grain borer, *Rhyzopertha dominica* (F.), to be extremely susceptible to spinosad (Fang et al. 2002a,b; Flinn et al. 2004, Huang et al. 2004, Subramanyam et al. 2007). The adults of sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.), and red flour beetle, *Tribolium castaneum* (Herbst), are moderately susceptible to spinosad (Fang et al. 2002a, Subramanyam et al. 1999, Nayak et al. 2005), but the first instars of these species are highly susceptible to spinsoad as evidenced by lack of progeny production on spinosad-treated grain (Toews and Subramanyam 2003, Flinn et al. 2004, Athanassiou et al. 2010*).*

Chlorpyrifos-methyl plus deltamethrin at the labeled rate of 3 plus 0.5 mg(AI)/kg is effective against several stored-grain psocids on stored wheat (Athanassiou et al. 2009). It is also effective against *R. dominica*, *T. castaneum*, the rice weevil, *Sitophilus oryzae* (L.); and Indianmeal moth, *Plodia interpunctella* (Hübner) on stored wheat and *R. dominica* and *S. oryzae* on short-grain rice and long-grain rice (Subramanyam et al. 2012).

Only two studies examined the effectiveness of spinosad against field strains of stored-grain insects. Spinosad was tested against two strains each of *R. dominica*, *T. castaneum*, and *P. interpunctella*, and one strain of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) collected from bins of stored hard red winter wheat in Kansas (Huang et al. 2004), and six strains of the confused flour beetle, *Tribolium confusum* Jacquelin du Val collected from different

countries of Europe (Athanassiou et al. 2008). In general, these studies showed field strains to be less susceptible than corresponding laboratory-reared strains. To our knowledge, field strains of stored-product insects have not been tested with chlorpyrifos-methyl plus deltamethrin. Field strains of insects may differ widely in their susceptibility to insecticide applied to grains due to natural tolerance or resistance (Subramanyam et al. 1989, Subramanyam and Hagstrum 1996, Huang et al. 2004, Kljajic and Peric 2006, Vayias et al. 2009). Therefore, evaluation of insecticides against field strains is necessary to confirm whether or not an approved insecticide at the labeled rates will work in practical field situations. In the present investigation, we determined susceptibility of adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* field strains collected mostly from farm-stored grain in Kansas to spinosad and chlorpyrifos-methyl plus deltamethrin applied to hard red winter wheat. Data on effectiveness of an insecticide against field strains are needed to make a recommendation for its use by farmers and grain managers; in addition such data provide baseline information on insect susceptibility which is important in pest management and resistance management programs.

Materials and Methods

Collection of Field Strains. Adults of *T. castaneum*, *O. surinamensis*, and *R. dominica* were collected from farm bins at cooperating farm sites (Table 1.2) between July and November 2011 by inserting five perforated probe traps (Subramanyam et al.1993) just below the stored grain surface to capture live adults. The traps were removed after 1 to 2 weeks. Additionally, 1 to 2 kg sample of mostly wheat, and some corn and sorghum were collected in 37.5 x 30.5 cm plastic Ziploc® bags (Assorted Bag Company, Dallas, TX). In the laboratory, live adults of each species (~50 to 200) were separated from grains. In addition, four strains of *T. castaneum* and one strain of *R. dominica* collected from flour mills in the United States prior to 2011, and one strain of *T. castaneum* collected in 2011 from a rice-processing facility were also included in this study. In total, 11 field strains of *T. castaneum*, six strains of *O. surinamensis*, and two strains of *R. dominica* were used in the study, along with the corresponding laboratory strains of each species, that have been in rearing since 1999 in the Department of Grain Science and Industry, Kansas State University.

Insect Rearing. Laboratory and field strains were reared on standard diets in a growth chamber at 28°C and 65% RH. Organic white wheat flour (Heartland Mills, Marienthal, KS) plus 5% (by wt) brewer's yeast diet was used for rearing *T. castaneum*. Clean, organic hard red winter wheat (Heartland Mills, Marienthal, KS) and rolled oats (Heartland Mills, Marienthal, KS) plus 5% brewer's yeast diet were used for rearing *R. dominica* and *O. surinamensis*, respectively.

Insecticides. The liquid formulations of spinosad and chlorpyrifos-methyl plus deltamethrin were supplied by Bayer CropScience (Research Traingle Park, Raleigh, NC). Liquid spinosad formulation (Contain II) had a purity of 232 mg (AI)/ml and the purity of chlorpyrifos-methyl plus deltamethrin (Storcide™ II) was 216 mg (AI)/ml of chlorpyrifos-methyl and 37 mg (AI)/ml of deltamethrin. Stock solutions and dilutions for grain treatment were made in distilled water.

Wheat Treatment. Organic, hard red winter wheat (Heartland Mills) was cleaned manually by sieving it over a 2-mm round hole sieve (Hogentogler and Co. Inc., Columbia, MD) to remove the dockage and broken kernels. After cleaning wheat was frozen for 1 wk at -13°C to kill any live insects. The moisture content of grain samples was equilibrated to 12 ± 1% in

environmental growth chambers maintained at 28°C and 65% RH and quantified with a Moisture Analyzer Model 930 (Shore Sales Co., Rantoul, IL).

Tests at Labeled Rates. Spinosad and chlorpyrifos-methyl plus deltamethrin were evaluated at their respective labeled rates of 1 mg(AI)/kg and 3 plus 0.5 mg(AI)/kg against laboratory and field strains of *T. castaneum*, *O. surinamensis*, and *R. dominica*.

Spinosad or chlorpyrifos-methyl plus deltamethrin solution (1 ml) was applied to 1 kg lots of wheat to provide the respective labeled rates. Each lot of wheat was treated with an insecticides in a 5 kg capacity stainless steel drum which was rotated mechanically for 10 min to ensure uniform coverage of the insecticides on the kernels. Wheat treated with aliquots of distilled water served as the control (0 mg [AI]/kg). Treated wheat (50 g) was weighed in separate 495 ml glass jars and 25 unsexed 1to 3 wk old adults of the test insect species were introduced into each jar. The jars after adult introduction were closed with metal lids fitted with wire mesh screens and filter papers and kept in the environmental chamber at 28°C and 65% RH.

The mortality of introduced adults was examined at 7 and 14 d, and adult progeny production was assessed after 42 d. An adult unable to move when prodded with a fine brush was considered dead. Each combination of insecticide, observation time, and insect species was replicated five times and each replicate was treated separately as explained above. The original number of introduced adults (25) was subtracted from the number of adult progeny produced prior to subjecting data to statistical analysis.

Dose-Response Bioassays. Initially bioassays were carried out on laboratory strains for standardization of doses to be used in dose-response bioassays only with spinosad, because laboratory and field strains of the three species exposed to chlorpyrifos-methyl plus deltamethrin at the labeled rate showed complete mortality and progeny suppression. Based on the tests at labeled rates, two least susceptible field strains of each species and the laboratory were selected for dose-response bioassays with spinosad. The doses of spinosad used were 0, 1, 2, 5, 10, 15, 20, 25, 30, 35 and 40 mg (AI)/kg for *T. castaneum*; 0, 0.2, 0.5, 0.7, 1 and 2 mg (AI)/kg for *O. surinamensis*; and 0, 0.005, 0.007, 0.01, 0.02, 0.03, 0.04, 0.06 mg (AI)/kg for *R. dominica*. Only for the field strains of *O. surinamensis*, rates of 5, 7 and 10 mg (AI)/kg were used in addition to 1 and 2 mg (AI)/kg.

Wheat (50 g) was taken in separate 495 ml glass jars and 50 µl of each insecticide solution was applied to obtain the desired insecticide rate. After adding the insecticide solution,

wheat in the glass jars was shaken by hand for 1 min to facilitate insecticide coverage on the grains. Wheat treated with aliquots of distilled water served as the control treatment (0 mg [AI]/kg). Twenty-five unsexed, 1 to 3 wk old adults of the test insect species were introduced into each jar. The jars were then closed with metal lids fitted with wire-mesh screens and filter papers and kept in the environmental chamber at 28ºC and 65% RH. Each combination of insecticide and rate for an insect species was replicated three times and each replication was treated separately. After 7 d, the grain samples were sieved and the number of live and dead adults counted. The contents (wheat and the insects) were transferred back into the jars and were kept back in the growth chamber to determine progeny production at 42 d post-infestation.

Data Analysis. In tests at labeled rates, the number of dead insects out of the total exposed to untreated and insecticide-treated wheat after 7 and 14 d were calculated as a percentage. Mortality data on insecticide-treated wheat were corrected for mortality on untreated (control) wheat (Abbott 1925). Corrected mortality data were transformed to angular values (Zar 1984) and adult progeny production data were transformed to $\log_{10}(x+1)$ scale for statistical analysis. Mortality data by species and insecticide were subjected to two-way analysis of variance (ANOVA) to determine difference in mortality between 7 and 14 d and among the strains. To determine differences among strains, corrected mortality data at 7 or 14 d were subjected to one-way ANOVA and means were separated using Bonferroni test at $\alpha = 0.05$ (SAS Institute 2008). The adult progeny produced on the untreated and spinosad-treated wheat for each insect species and strain were compared using two-sample *t*-tests (SAS Institute 2008), because of wide variation in progeny production among strains.

The mean mortality data from dose-response tests for each insect species on spinosad-treated wheat were corrected for mortality on untreated wheat (Abbott 1925). Corrected dose-mortality data were subjected to probit analysis (SAS Institute 2008) for determining the dose producing 50% (LD_{50}) and 99% (LD_{99}) mortality of insects and associated statistics. The LD_{99} values for any two strains of a species were compared using a ratio test (Robertson and Preisler 1992). The percent reduction in progeny production in the spinosad-treated wheat relative to the control treatment was calculated. These data were subjected to probit analysis, where possible, to determine effective dose for 50% (ED_{50}) and 99% (ED_{99}) reduction in progeny production (SAS Institute 2008). The ED_{99} values for any two strains of a species were compared using a ratio test. The LD_{99} or ED_{99} values between any two strains were considered significantly different (*P*

< 0.05) if the 95% confidence interval for the ratio did not include 1 (Robertson and Preisler 1992).

Results

Tests at Labeled Rates. The 7 d and 14 d mortality of all *T. castaneum* strains ranged on untreated wheat ranged from 0 to 4% and from 0 to 9%, respectively. The 7 and 14 d mortality of all the strains on spinosad-treated wheat ranged from 2 to 18% and 4 to 58%, respectively (Table 2.1). The two-way ANOVA by insect species and insecticide showed that the 14-d mortality of *T. castaneum* strains when exposed to spinosad-treated wheat was greater than 7-d mortality *(F* = 79.7; df = 1, 96; $P < 0.0001$). Mortality differences among strains were also significant ($F = 6.3$; df = 11, 96; $P < 0.0001$) as was the interaction of strain and day ($F = 3.5$; df = 11, 96; $P = 0.0004$). One way ANOVA showed significant differences among strains for mortality data collected on day 7 ($F = 3.1$; df = 11, 48; $P = 0.0032$) and day 14 ($F = 6.0$; df = 11, 48; $P < 0.0001$). The 7 d mortality of CF strain was significantly different ($P \leq 0.05$) from that of TP, whereas no differences were observed among all other strains. A similar trend was observed for the 14 d mortality among strains. The CF strain mortality was significantly different ($P \leq 0.05$) from that of the AB1 strain, and despite wide variation in mortality among strains, significant differences were not detected. Chlorpyrifos-methyl plus deltamethrin was extremely effective against all *T. castaneum* strains with 100% mortality on both 7 d and 14 d.

Adult progeny production among *T. castaneum* strains in the control treatment ranged from 10 to 86 adults/jar (Table 2.2). There was no progeny production in four out of the 11 field strains exposed to spinosad-treated wheat. Except for the CF strain, reduction in progeny production relative to the corresponding control treatment ranged from 90 to 100%. In six strains, where minimal progeny production was observed, the numbers produced were significantly lower ($P \leq 0.05$) than in the corresponding control treatment. The progeny production of the CF strain exposed to spinosad was similar ($P \geq 0.05$) to production of the same strain on untreated wheat. None of the strains exposed to chlorpyrifos-methyl plus deltamethrin produced progeny.

The mean 7 d and 14 d mortality of *O. surinamensis* strains on untreated wheat was less than 2 and 9%, respectively. All six field strains of *O. surinamensis* after 7 to 14 d exposure were

less susceptible to spinosad (4 to 20% mortality) when compared with the laboratory strain (77 to 80% mortality) (Table 2.3). There were significant differences in mortality among the strains (F = 62.2; df = 6, 56; $P < 0.0001$) but mortality was similar after 7 and 14 d of exposure (F = 3.1; df = 1, 56; P = 0.085). The strain and exposure time interaction was not significant (F = 0.92; df = 6, 56; P = 0.488). One-way ANOVA showed differences among strains at both 7 (F = 36.1; df = 6, 28; $P < 0.0001$) and 14 d (F = 27.4; df = 6, 28; $P < 0.0001$). The significant effect observed is due to mortality of the laboratory strain being higher than the field strains. The 7 d mortality of all field strains was similar ($P \geq 0.05$), whereas the 14 d mortality of strain AB2 was different ($P \leq 0.05$) from that of CF strain but was not different ($P \geq 0.05$) among all other field strains. Exposure to chlorpyrifos-methyl plus deltamethrin for 14 d resulted in complete mortality of five of the six *O. surinamensis* field strains and the laboratory strain. The 7 d mortality of five strains was 100% and that of other two field strains was 84 to 96%. Mortality differences were observed among strains (F= 19.5; df = 6, 56; $P < 0.0001$) but not between 7 and 14 d (F= 3.5; df = 1, 56; P = 0.066). The strain and exposure time interaction also was not significant (F = 1.62; df = 6, 56; P = 0.159). One-way ANOVA showed mortality among strains at 7 (F = 9.8; df = 6, 28; $P <$ 0.001) and 14 d (F = 13.7; df = 6, 28; $P < 0.0001$) to be significantly different. This difference was primarily due to AB1 strain showing significantly lower ($P \leq 0.05$) mortality than the other strains.

Adult progeny production of each *O. surinamensis* strain was significantly lower ($P \leq$ 0.05) on spinosad-treated wheat when compared with production on untreated wheat (Table 2.4). Percent reduction in progeny production on spinosad-treated grain relative to that on untreated wheat ranged from 68 to 89%. No progeny were produced in strains when exposed to chlorpyrifos-methyl plus deltamethrin.

The mean 7 d and 14 d mortality of *R. dominica* strains in control treatment was less than 5% and 10%, respectively. Spinosad was extremely effective against all *R. dominica* strains (F = 1.0; df = 2, 24; P = 0.383) with 99 to 100% mortality after 7 and 14 d. Therefore, difference among strains (F = 1.0; df = 2, 24; P = 0.383) and between exposure times (F = 1.0; df = 1, 24; P = 0.327) were not significant (Table 2.5). The strain and exposure time interaction also was not significant (F= 1.0; df = 2, 24; P = 0.383).

Like spinosad, chlorpyrifos-methyl plus deltamethrin was extremely effective against *R. dominica*. There were no significant differences among strains (F = 2.67; df = 2, 24; P = 0.090)

and between the exposure times (F = 2.67; df = 1, 24; P = 0.116). The strain and exposure time interaction was not significant (F = 2.67; df = 2, 24; P = 0.09). Adult progeny production of *R. dominica* on the untreated wheat for the laboratory strain was 357 adults/jar, whereas for the CF and RL strains, it was 35 and 36 adults/jar, respectively. No progeny production was observed in strains exposed to either spinosad or chlorpyrifos-methyl plus deltamethrin.

Dose-Response Tests. The LD_{50} and LD_{99} values and associated statistics for the *T. castaneum*, *O. surinamensis*, and *R. dominica* strains exposed to spinosad are shown in Table 2.6. Based on LD_{50} and LD_{99} values, the adults of *R. dominica*, in general, were highly susceptible to spinosad followed by *O. surinamensis* and *T. castaneum*. Ratio tests using the LD_{99} values showed that there were no differences ($P > 0.05$) between the three strains of *T. castaneum* or three strains of *R. dominica* (Table 2.7). The two field strains of *O. surinamensis* were not significantly different from one another ($P > 0.05$), but each field strain had significantly higher ($P < 0.05$) LD_{99} than the corresponding laboratory strain.

Adult progeny production of *T. castaneum* on the untreated wheat was 55 adults/jar, and that of the CF and HN strains was 52 and 61 adults/jar, respectively. Progeny production was observed only with the two field strains exposed to 1 mg(AI)/kg of spinosad; the CF strain produced 8 adults/jar while the HN strain produced 2 adults/jar. Reduction in progeny production at 1 mg(AI)/kg of these strains compared with production of the same strains on untreated wheat was 87 to 96%.

The laboratory strain of *O. surinamensis* failed to produce adult progeny at spinosad dosages of 2 mg(AI)/kg and above, but produced progeny at 0, 0.2, 0.5, 0.7, and 1.0 mg(AI)/kg of spinosad (Table 2.8). Even at the labeled rate of 1.0 mg(AI)/kg, the reduction in progeny production of the laboratory strain when compared with production on untreated wheat was 76%. The progeny production on untreated wheat was approximately similar among the laboratory and the two field strains. The field strains did not produce progeny at spinosad dosages of 7 to 20 mg(AI)/kg, but those exposed to 1, 2, and 5 mg(AI)/kg produced progeny. In the two strains, reduction in progeny production on spinosad-treated wheat was 57 to 97% relative to production of the same strains on untreated wheat. The ED_{99} values for progeny reduction were six to eight times higher than the ED_{50} values (Table 2.9), and in general, for the field strains the spinosad dose for 50 and 99% progeny reduction was slightly lower than the dose required to produce 50 and 99% adult mortality. The ED_{99} values for progeny production of

the AB1 and AB2 strain were not significantly different from one another ($P < 0.05$) based on the ratio test (ratio [95% CL] was 1.39 [0.81 - 2.38]). Each of the field strains, AB1 (1.89 [0.88 - 4.07]) and AB2 (1.36 [0.64 - 2.89]), were also not different from the laboratory strain.

Adult progeny production of the laboratory and two field strains of *R. dominica* on untreated wheat ranged from 176 to 254 adults/jar (Table 2.10). Progeny production was inversely related to spinosad rate for all strains. At the highest spinosad dose (0.06 mg(AI)/kg, the laboratory strain failed to produce any progeny but eight to 10 adults/jar were observed in the two field strains. The ED_{99} values for progeny reduction of *R. dominica* strains were 7 to 15 times higher than the corresponding ED_{50} values (Table 2.11). The ED_{99} values for progeny reduction of the three strains was ≤0.1 mg(AI) of spinosad/kg of grain. Comparison of the ED_{99} values for the CF and RL field strains using the ratio test showed that they were not significantly different ($P > 0.05$) from one another (1.40 [0.78 - 2.50]. The ED_{99} value of the CF strain was significantly different ($P < 0.05$) from that of the laboratory strain (2.22 [1.38 - 3.57]), but the ED_{99} value of RL and laboratory strains were not different ($P > 0.05$) from one another (1.59 [0.93 - 2.71]).

Discussion

Spinosad is one of the promising grain protectants (Hertlein et al. 2011), and has not been commercially released yet. In the present study, a 32-fold variation in adult mortality among *T. castaneum strains* and 6-fold variation among *O. surinamensis* strains was observed when exposed to spinosad-treated wheat. Variation in susceptibility among different insects and different strains of the same insect species to an insecticide is common (Subramanyam et al. 1989, Subramanyam and Hagstrum 1996, Kljajic and Peric 2006). Huang et al. (2004) reported the field strains of *T. castaneum*, *P. interpunctella*, and *C. ferrugineus* were two to six times less susceptible to spinosad than the corresponding laboratory strains based on the LD_{95} values. The strains of *T. confusum* from different locations in Europe differed markedly in susceptibility to spinosad with adult mortality ranging from 1 to 81% after 7 d exposure on wheat treated with spinosad dust at 0.19 mg(AI)/kg (Athanassiou et al. 2008). The large variation in susceptibility among strains may be due to differences in mobility, insecticide pick-up, penetration, and metabolism.

In our study, both the laboratory and field strains of *T. castaneum* were less susceptible to spinosad even at rates much higher than the labeled rate. Previous studies have shown *T. castaneum* adults to be less susceptible to spinosad on wheat (Fang et al. 2002a, b; Flinn et al. 2004, Subramanyam et al. 2007). However, susceptibility of *T. castaneum* adults to spinosad at 1 mg(AI)/kg varies with the wheat class with 14 d mortality ranging from 2 to 55% on hard red spring, soft red winter, hard red winter and durum wheats (Fang et al. 2002a). Our results showed that even though there was wide variation in adult mortality among strains, there was 90 to 100% reduction in progeny production relative to corresponding control treatment except in the CF strain. The low adult progeny production on spinosad-treated wheat in 10 out of the 11 field strains indicated that the neonates probably were highly susceptible to spinosad. Toews and Subramanyam (2003) reported 100% suppression of egg-to-adult emergence of *T. castaneum* exposed as eggs to whole hard red winter wheat treated with spinosad at the labeled rate after 14 d of exposure. Athanassiou et al. (2008) evaluated the efficacy of spinosad dust against six *T. confusum* strains from different locations of Europe and found that larvae (3–4th instar) were generally more susceptible than adults for all strains tested. The high progeny production in the

CF strain may be an anomaly and not an indicator of resistance since the LD_{99} value of this strain was similar to that of the laboratory strain in dose-response tests.

The field strains of *O. surinamensis* showed reduced susceptibility to spinosad when compared with the laboratory strain. This was confirmed by dose-response bioassays as the LD_{99} values of the two field strains were about six times higher than that of the laboratory strain. However, the progeny production was not adversely affected as shown by similar ED_{99} values of the laboratory and field strains. Therefore, there may be natural tolerance or resistance in *O. surinamensis* field strains with no reproductive disadvantage. Complete progeny suppression in *O. surinamensis* could not be achieved which may be due to lower susceptibility of immatures to spinosad. Toews and Subramanyam (2003) reported that the 14 d egg-to-adult suppression of *O. surinamensis* exposed as eggs to labeled rate of spinosad on whole wheat was about 80%, indicating reduced susceptibility of neonates. Effectiveness of spinosad against *O. surinamensis* varies depending on the type of commodity. Mortality of *O. surinamensis* adults ranged from 3 to 76% among different classes of wheat treated with spinosad at the labeled rate after 7 and 14 d exposure. Spinosad was effective against *O. surinamensis* only on durum wheat (Fang et al. 2002a). Subramanyam et al. (1999) also found *O. surinamensis* to be moderately susceptible to spinosad on hard red spring wheat based on adult mortality, and significant reduction in adult progeny production only at rates $\geq$ 3 mg(AI)/kg. Spinosad was highly effective against *O. surinamensis* on corn with >98% mortality after 12 days of exposure and complete progeny suppression (Huang and Subramanyam 2007). Nayak et al. (2005) reported that the adult mortality of malathion and fenitrothion resistant *O. surinamensis* strain after 7, 14 or 42 d exposure to wheat treated with spinosad at the labeled rate was $\leq$ 33% and not significantly different from the control. Daglish et al. (2003) found that the adult mortality of *O. surinamensis* was <10% after 14 d of exposure on spinosad-treated wheat with only 7% reduction in progeny production.

Spinosad was highly effective against *R. dominica* adults of all strains as evidenced by complete mortality within 7 d and complete progeny suppression. These findings are consistent with previous studies which also have shown high susceptibility of *R. dominica* to spinosad at a rate as low as 0.1 mg(AI)/kg (Fang et al. 2002a, b; Flinn et al. 2004, Huang et al. 2004, Getchell and Subramanyam 2008, Subramanyam et al. 2012). Daglish and Nayak (2006) also found that spinosad applied at 0.5 or 1 mg(AI)/kg was completely effective against *R. dominica* for nine

months with 100% adult mortality and progeny suppression. Nayak et al. (2005) reported that spinosad was very effective against organophosphate-resistant *R. dominica* populations. Delayed mortality effects after short exposures to spinosad have been reported in *R. dominica* adults (Getchell and Subramanyam 2008, Boina et al. 2012), and contribute to spinosad's overall effectiveness against this species.

Chlorpyrifos-methyl plus deltamethrin was by far a very effective protectant on wheat, with practically no variation in efficacy among strains of *T. castaneum* and *R. dominica,* given that 100% or close to 100% adult mortality was recorded after only 7 d of exposure with total progeny suppression. It was extremely effective against five of the six field strains of *O. surinamensis* with complete mortality and progeny suppression. Lack of progeny production in chlorpyrifos-methyl plus deltamethrin treatment could be due to rapid mortality of introduced adults before they have had a chance to lay eggs or high susceptibility of first instars hatching from eggs laid by the adults. Chlorpyrifos-methyl plus deltamethrin was effective against psocid species *Lepinotus reticulatus* Enderlein, *Liposcelis entomophila* (Enderlein), *L. bostrychophila* Badonnel and *L. paeta* Pearman in rice and wheat (Athanassiou et al. 2009). It was also effective against *R. dominica*, *S. oryzae* and *T. castaneum* on wheat (Subramanyam et al. 2012). The combined treatment of chlorpyrifos-methyl at 6 mg(AI)/kg and deltamethrin at 0.5 or 1 mg(AI)/kg resulted in 100% mortality of *R. dominica* and *S. oryzae* on wheat and *T. castaneum* on corn (Arthur 1994). Application of deltamethrin (0.5 mg[AI]/kg) with chlorpyrifos-methyl (8 mg[AI]/kg) provided complete protection for at least 9 months against the larger grain borer, *Prostephanus truncatus* (Horn) and *Sitophilus* spp. in shelled maize stored in galvanized steel bins or jute bag stacks in Tanzania (Dales and Golob 1997).

There are several reports of resistance to chlorpyrifos-methyl in the field strains of *R. dominica* (Zettler and Cuperus 1990, Beeman and Wright 1990, Guedes et al. 1996). Subramanyam et al. (2007) reported that chlorpyrifos-methyl at 3 mg(AI)/kg was only partially effective against *R. dominica* but effective against *T. castaneum* on stored wheat. The mean number of live adults of *R. dominica* found in wheat treated with chlorpyrifos-methyl at 3 mg(AI)/kg increased from 0 to 10 adults/kg at the end of the six-month study period. Laboratory bioassays with *R. dominica* adults from chlorpyrifos-methyl treated wheat also confirmed poor activity (Subramanyam et al. 2007). Therefore, the enhanced effectiveness of chlorpyrifos-methyl plus deltamethrin against *R. dominica* can be attributed to deltamethrin in this

combination product. Arthur (1994) also reported that deltamethrin applied at 0.5, 0.75, or 1 mg(AI)/kg on wheat was effective against *R. dominica* for at least 10 months. Deltamethrin at 0.125 or 0.375 mg(AI)/kg synergized with 2.5 mg(AI)/kg of piperonyl butoxide controlled *R. dominica* on wheat for six months (Nicolas et al. 1991). Deltamethrin emulsifiable concentrate formulation applied at 0.25 mg(AI)/kg was found to be effective on stored wheat against *T. confusum* (Athanassiou et al. 2004b) and *S. oryzae* (Athanassiou et al. 2004a) after 7 d of exposure, with high mortality and production of only a few adult progeny. Chlorpyrifos-methyl plus deltamethrin is a relatively new product and it is plausible that the strains of *R. dominica* from Kansas may not have developed resistance to deltamethrin.

Our study provides baseline data on susceptibility of laboratory and field strains of three stored-grain insect species to two of the insecticides approved as grain protectants. This information would be helpful in screening changes in susceptibility of these species to insecticides in Kansas, once these insecticides are used in the field on a continuous basis. Baseline data generated here can be used to detect cases of incipient resistance. Additional work is needed to determine the behavioural or physiological factors responsible for the differential performance of spinosad among strains. In summary, our results show that chlorpyrifos-methyl plus deltamethrin is an ideal insecticide to use on stored wheat to control adults of *R. dominica*, *T. castaneum* and *O. surinamensis* strains and for suppression of progeny production. However, continuous use of this combination product may lead to resistance to chlorpyrifos-methyl or deltamethrin as documented by various researchers worldwide (Beeman and Wright 1990, Zettler and Cuperus 1990, Collins et al. 1993, Guedes et al. 1996, Lorini and Galley 1999, Perez-Mendoza 1999, Ribeiro et al. 2003). Therefore, it is advisable to use the combination product in rotation with spinosad or other approved grain protectants (Arthur and Subramanyam 2012) as part of an integrated insect pest management and resistance management program.

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Table 2.1 Corrected mortality of adults of *T. castaneum* strains exposed for 7 and 14 days to wheat treated with labeled rates of spinosad and chlorpyrifos-methyl (C-methyl) plus deltamethrin

Strain	Mean ± SE (%) mortality[a]			
	Spinosad		C-methyl + deltamethrin	
	7 d[b]	14 d[b]	7 d	14 d
Lab.	4.8 ± 0.8ab	35.5 ± 2.6ab	100	100
AB1	12.3 ± 2.5ab	57.7 ± 8.3a	100	100
CF	1.8 ± 1.3b	4.3 ± 3.5c	100	100
CN	14.0 ± 4.2ab	25.3 ± 3.0abc	100	100
HN	7.2 ± 4.3ab	23.2 ± 6.0abc	100	100
MN1	2.1 ± 1.0ab	43.4 ± 3.5ab	100	100
PD1	5.6 ± 2.4ab	25.6 ± 10.5bc	100	100
SR	11.2 ± 3.2ab	28.8 ± 5.3ab	100	100
TP	17.8 ± 4.3a	24.4 ± 7.9abc	100	100
KC	8.0 ± 4.4ab	21.6 ± 7.0bc	100	100
IL	11.2 ± 1.5ab	13.7 ± 3.6bc	100	100
AZ	5.6 ± 2.0ab	11.2 ± 2.3bc	100	100

[a]Each mean is based on $n = 5$.

Mean 7 d mortality in control treatment in all strains ranged from 0.0 ± 0.0 to 4.4 ± 1.8% and 14 d mortality from 0.0 ± 0.0 to 8.8 ± 4.6%.

[b]Means by day among strains followed by different letters are significantly different ($P < 0.05$; by Bonferroni *t*-test).

Table 2.2 Adult progeny production of *T. castaneum* strains exposed continuously for 42 days to labeled rates of spinosad on wheat[a]

Strain	Mean ± SE Progeny (% reduction[b])		*t* (df)	*P*-value
	Control	Spinosad		
Lab.	39.2 ± 5.5	0 (100.0)	____[c]	____
AB1	10.4 ± 2.3	0 (100.0)	____	____
CF	69.2 ± 19.8	40.4 ± 4.2 (41.6)	-1.38 (8)[d]	0.205
CN	30.2 ± 12.4	0.2 ± 0.2 (99.3)	-2.84 (4.22)[e]	0.044*
HN	46.2 ± 2.7	0 (100.0)	____	____
MN1	10.2 ± 2.7	0 (100.0)	____	____
PD1	15.4 ± 6.9	0.6 ± 0.4 (96.1)	-2.74 (8)[d]	0.026*
SR	41.2 ± 1.7	0 (100.0)	____	____
TP	86.2 ± 5.5	8.8 ± 2.0 (89.8)	-9.98 (4.57)[e]	0.0003*
KC	72.2 ± 3.9	0.4 ± 0.2 (99.4)	-22.51 (4.8)[e]	<0.0001*
IL	78.4 ± 4.3	2.4 ± 0.7 (96.9)	-14.86 (4.56)[e]	<0.0001*
AZ	75.0 ± 10.4	7.2 ± 1.7 (90.4)	-8.00 (8)[d]	<0.0001*

[a]None of the strains produced adult progeny in chlorpyrifos-methyl plus deltamethrin treatment.

[b]Percent reduction in adult progeny production of field strains relative to the corresponding control treatment.

[c]Data were not subjected to two sample *t*-test because no progeny was produced in spinosad treatments.

[d]Variances were equal, *F*, range = 1.69 – 7.82; df = 4,4; *P*, range = 0.071 – 0.623.

[e]Variances were unequal, *F*, range = 9.93 – 37.07; df = 4,4; *P*, range = 0.004 – 0.047.

*Significant ($P \leq 0.05$).

Table 2.3 Corrected mortality of adults of *O. surinamensis* strains exposed for 7 and 14 days to wheat treated with labeled rates of spinosad and chlorpyrifos-methyl (C-methyl) plus deltamethrin

Strain	Mean ± SE mortality (%)[a]			
	Spinosad		C-methyl + deltamethrin	
	7 d[b]	14 d[b]	7 d[b]	14 d[c]
Lab.	80.2 ± 2.0a	77.1 ± 4.5a	100a	100
AB1	7.2 ± 2.0b	10.0 ± 2.5bc	83.7 ± 5.9b	93.6 ± 2.0
AB2	4.0 ± 0.0b	3.5 ± 1.2c	96.0 ± 4.0a	100
CF	9.6 ± 2.0b	19.5 ± 5.0b	100a	100
MN1	9.7 ± 1.6b	16.9 ± 3.4bc	100a	100
PD1	4.8 ± 1.5b	13.0 ± 3.8bc	100a	100
TP	20.0 ± 6.6b	16.9 ± 2.5bc	100a	100

[a]Each mean is based on $n = 5$.

Mean 7 d and 14 d mortality in the control treatment ranged from 0.0 ± 0.0 to 1.7 ± 1.7 and from 0.0 ± 0.0 to 8.6 ± 1.4%, respectively.

[b]Means for each insecticide by day among insect strains followed by different letters are significantly different ($P < 0.05$; by Bonferroni *t*-test).

[c]One-way ANOVA was significant (F-value = 13.67; df = 6, 28; $P < 0.0001$).

Table 2.4 Adult progeny production of *O. surinamensis* strains exposed continuously for 42 days to labeled rates of spinosad on wheat[a]

Strain	Mean ± SE adult progeny (% reduction[b])		*t* (df)	*P*-value*
	Control	Spinosad		
Lab.	96.0 ± 5.47	10.2 ± 3.7 (89.4)	-8.97 (4.39)[d]	0.0005
AB1	70.6 ± 10.2	21.2 ± 2.5 (70.0)	-6.37 (8)[c]	0.0002
AB2	98.4 ± 2.3	31.2 ± 4.9 (68.3)	-8.50 (4.24)[d]	0.0008
CF	79.0 ± 6.7	11.0 ± 3.6 (86.1)	-7.28 (4.81)[d]	0.0009
MN1	66.2 ± 5.1	7.2 ± 0.9 (89.1)	-16.42 (8)[c]	<.0001
PD1	112.4 ± 9.2	31.8 ± 4.0 (71.7)	-8.42 (8)[c]	<.0001
TP	38.0 ± 2.2	7.0 ± 1.1 (81.6)	-10.32 (8)[c]	<.0001

[a]There were no adult progeny produced by all strains in chlorpyrifos-methyl plus deltamethrin treatment except AB1 strain where 0.2 ± 0.2 adults/jar were produced.

[b]Percent reduction in adult progeny production of field strains relative to the corresponding control treatment.

[c]Variances were equal, *F*, range = 1.67 – 6.29; df = 4, 4; *P*, range = 0.103 – 0.633.

[d]Variances were unequal, *F*, range = 9.77 – 33.19; df = 4, 4; *P*, range = 0.005 – 0.049.

*All *P*-values were significant ($P \leq 0.05$).

Table 2.5 Corrected mortality of adults of *R. dominica* strains exposed for 7 and 14 days to wheat treated with labeled rates of spinosad and chlorpyrifos-methyl (C-methyl) plus deltamethrin

Strain	Mean ± SE mortality (%)[a]			
	Spinosad		C-methyl + deltamethrin	
	7 d	14 d	7 d	14 d
Lab.	100	100	100	100
CF	100	99.2 ± 0.8	98.3 ± 1.0	100
RL	100	100	100	100

[a]Each mean is based on $n = 5$.

Mean 7 d and 14 d mortality strains in control treatment ranged from 0.0 ± 0.0 to 5.0 ± 1.6% and from 0.0 ± 0.0 to 10.4 ± 2.7%, respectively.

Table 2.6 Probit regression estimates for laboratory and field strains of three insect species exposed to spinosad- treated wheat

Species	Strain	N^a	Mean ± SE		LD (95% CL) (mg/kg)		χ^2 (df)	*P*-value*
			Intercept	Slope	LD_{50}	LD_{99}		
T. castaneum[b]	Lab	975	-0.97 ± 0.21	1.93 ± 0.21	3.17 (2.26 - 4.14)	51.0 (33.51 - 94.57)	5.15 (9)	0.821
	CF	900	-2.42 ± 0.40	2.88 ± 0.35	6.92 (5.24 - 8.49)	44.56 (33.27 - 69.32)	7.94 (8)	0.440
	HN	825	-1.89 ± 0.27	2.60 ± 0.26	5.31 (4.09 - 6.66)	41.70 (29.69 - 66.95)	2.79 (7)	0.904
O. surinamensis[b]	Lab	525	0.99 ± 0.21	5.04 ± 0.92	0.64 (0.54 - 0.73)	1.84 (1.39 - 3.26)	1.20 (3)	0.753
	AB1	600	-1.08 ± 0.25	3.14 ± 0.43	2.21 (1.71 - 2.73)	12.16 (8.49 - 21.79)	4.11 (4)	0.392
	AB2	600	-1.93 ± 0.33	4.24 ± 0.56	2.86 (2.35 - 3.42)	10.12 (7.62 - 15.79)	1.42 (4)	0.842
R. dominica[c]	Lab	975	8.97 ± 0.97	4.54 ± 0.49	0.010 (0.009 - 0.012)	0.03 (0.027 - 0.048)	10.89 (9)	0.284
	CF	600	6.32 ± 0.92	3.24 ± 0.47	0.011 (0.009 - 0.014)	0.06 (0.04 - 0.12)	1.76 (4)	0.780
	RL	600	6.60 ± 0.98	3.35 ± 0.49	0.011 (0.009 - 0.013)	0.05 (0.04 - 0.11)	1.97 (4)	0.742

[a]Total number of insects used in the bioassays.

[b]Mortality of *T. castaneum and O. surinamensis* strains on untreated (control) wheat was 0%.

[c]Mean ± SE mortality of three *R. dominica* strains on untreated (control) wheat ranged from 2.7 ± 2.7 to 6.7 ± 6.7%.

*Goodness-of-fit of the probit model to dose/response data was not significant ($P > 0.05$), indicating good fit of the model to data.

Table 2.7 Comparison of LD_{99} values between field and corresponding laboratory strains of three stored-product insect species exposed to spinosad-treated wheat

Species	Strains compared[a]	LD_{99} ratio (95% CL)
T. castaneum	Lab. vs. CF	1.14 (0.62 - 2.13)
	Lab. vs. HN	1.22 (0.64 - 2.34)
O. surinamensis	AB1 vs. Lab.	6.60 (3.66 - 11.93)*
	AB2 vs. Lab.	5.49 (3.27 - 9.24)*
R. dominica	CF vs. Lab.	1.71 (0.96 - 3.07)
	RL vs. Lab.	1.54 (0.86 - 2.76)

[a]The strain mentioned first has the larger LD_{99} value in the pair being compared.

*The LD_{99} values within a pair being compared are significantly different ($P < 0.05$) from one another because the ratio does not include 1.

Table 2.8 Adult progeny production of *O. surinamensis* strains exposed for 42 days to various spinosad rates on wheat

Rate (mg[AI]/kg)	Mean ± SE Progeny (% reduction[a])		
	Lab.[b]	AB1[b]	AB2[b]
0	28.7 ± 3.2	29.7 ± 8.4	33.3 ± 6.6
0.2	23.7 ± 1.3 (17.5)	—[c]	—
0.5	20.0 ± 4.6 (30.3)	—	—
0.7	11.3 ± 3.8 (60.5)	—	—
1	7.0 ± 1.7 (75.6)	12.7 ± 2.0 (57.4)	13.3 ± 3.2 (60.0)
2	0 (100.0)	4.7 ± 2.6 (84.3)	4.0 ± 0.6 (88.0)
5	0 (100.0)	1.0 ± 0.6 (96.6)	0.3 ± 0.3 (99.0)

[a]Percent reduction in adult progeny production of each strain relative to the corresponding control treatment.

[b]For each strain at 7, 10, 15, and 20 mg(AI)/kg, no progeny were produced (100% reduction).

[c]Spinosad rates of 0.2 - 0.7 mg(AI)/kg were not tested on these strains.

Table 2.9 Probit regression estimates (mean ± SE) showing effective dose (ED) for progeny reduction for the laboratory and field strains of *O. surinamensis* exposed to spinosad- treated wheat

Strain	*N*[a]	Mean ± SE		ED (95% CL) (mg[AI]/kg)		χ^2 (df)	*P*-value
		Intercept	Slope	ED_{50}	ED_{99}		
Lab.	525	0.75 ± 0.19	2.95 ± 0.53	0.56 (0.36 - 0.79)	3.43 (1.83 - 20.23)	21.45 (4)	0.0003*
AB1	600	0.19 ± 0.12	2.63 ± 0.34	0.85 (0.64 -1.03)	6.49 (4.75 -10.72)	1.61 (2)	0.448
AB2	600	0.25 ± 0.12	3.11 ± 0.45	0.83 (0.63 - 0.99)	4.66 (3.50 - 7.61)	0.27 (2)	0.872

[a] Total number of insects used in the bioassays.

* Goodness-of-fit of the probit model to dose/response data was significant ($P < 0.05$), indicating poor fit of the model to data.

Table 2.10 Mean ± SE adult progeny production of *R. dominica* strains exposed for 42 d to various spinosad rates on wheat

Rate (mg[AI]/kg)	Mean ± SE Progeny (% reduction[a])		
	Lab.	CF	RL
0	180.3 ± 7.3	175.7 ± 3.9	254.3 ± 10.3
0.005	107.3 ± 11.3 (40.5)	121.7 ± 13.9 (30.8)	170.0 ± 37.5 (33.1)
0.007	84.7 ± 3.4(53.0)	94.7 ± 3.0 (46.1)	162.7 ± 41.9 (36.0)
0.01	62.3 ± 14.6 (65.4)	77.0 ± 14.0 (56.2)	133.3 ± 34.4 (47.6)
0.02	31.3 ± 5.9 (82.6)	41.3 ± 5.6 (76.5)	68.7 ± 4.3 (73.0)
0.03	7.3 ± 0.9 (95.9)	17.7 ± 2.7 (89.9)	10.3 ± 3.4 (95.9)
0.04	0.7 ± 0.7 (99.6)	15.3 ± 5.6 (91.3)	16.0 ± 0.6 (93.7)
0.06	0 (100.0)	10.3 ± 0.9 (94.1)	7.7 ± 4.2 (97.0)

[a]Percent reduction in adult progeny production of each strain relative to the corresponding control treatment.

Table 2.11 Probit regression estimates (mean ± SE) showing effective dose (ED) for progeny reduction for the laboratory and field strains of *R. dominica* exposed to spinosad-treated wheat

Strain	*N*[a]	Mean ± SE		ED (95% CL) (mg[AI]/kg)		χ^2 (df)	*P*-value
		Intercept	Slope	ED_{50}	ED_{99}		
Lab.	975	5.54 ± 0.42	2.55 ± 0.21	0.007 (0.006 - 0.008)	0.05 (0.04 - 0.08)	6.96 (5)	0.224
CF	600	4.16 ± 0.30	2.0 ± 0.16	0.008 (0.007 - 0.010)	0.12 (0.09 - 0.19)	1.94 (5)	0.858
RL	600	4.85 ± 0.49	2.38 ± 0.26	0.009 (0.007 - 0.011)	0.09 (0.055 - 0.189)	11.12 (5)	0.049*

[a] Total number of insects used in the bioassays.

*Goodness-of-fit of the probit model to dose/response data was significant ($P < 0.05$), indicating poor fit of the model to data.